OBSERVATIONS on the PATHOLOGY of the THYROID GLAND with special reference to AUTO-IMMUNITY.

by

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THESIS for degree of DOCTOR of PHILOSOPHY.

December, 1958.

ACKNOWLEDGEMENTS.

I wish to express my gratitude for valuable help willingly given by my colleagues.

Dr. W.S.A. Allan,
Pathology Department, University of Edinburgh.

Mr. J.A.L. Clark,
Consultant Surgeon, Stracathro Hospital.

Dr. E.K. Dawson,
Royal College of Physicians Laboratory,
Edinburgh.

Mr. T.C. Dodds,
Medical Photography Unit, University of
Edinburgh.

Mrs. D. Doniach,
Middlesex Hospital, London.

Miss Sheila Heath and Miss Joy Chowdray,
Pathology Department, University of Edinburgh.

Dr. A.G. Macgregor,
Royal Infirmary, Edinburgh.

Dr. M.K. MacDonald,
Pathology Department, University of Edinburgh.

Professor G.L. Montgomery,
Pathology Department, University of Edinburgh.

I am especially grateful to Professor G.L. Montgomery whose help made this work possible.

PREFACE.

The purpose of this thesis is to determine the frequency with which thyroglobulin antibodies are found in various thyroid diseases, to assess the value of serological methods in clinical diagnosis and relate the presence of thyroglobulin antibodies to histological appearances in excised glands.

INDEX.

INTRODUCTION.

THE CONCEPT OF AUTO-IMMUNITY AND ITS RELATION TO THYROID PATHOLOGY.

The term auto-immunity defines a state in which the host has produced antibodies to one or more of its own tissues. The idea is not new and as long ago as 1904 the French physician Chauffard introduced the term "immuno-haematology" in recognition of the presence of red cell antibodies in some forms of haemolytic anaemia. Pfeiffer (1956) recalls that Metchinikoff hinted at the possibility of auto-immunity although this was flatly rejected by Ehrlich who believed the organism could never form antibodies against itself and advanced the stern hypothesis of "horror intoxicatus." The physiological advantage of an immunity system which deals effectively with foreign unwanted material such as virus or bacteria is obvious and it seems both unnatural and improbable that the same system can unleash its capacity for destruction on a fellow organ be it liver, thyroid or nervous system.

The handling of foreign organic material is clearly the function of the reticulo-endothelial system whose characteristic reaction is phagocytosis and antibody production. At the same

time this system deals with worn out body tissue cells which are removed and digested without antibody formation. For example 1% of our circulating red cells are taken up each day by reticulo-endothelial system and no detectable circulating antibodies are produced for this task. This paradox has been recognised by Burnet (1954) who puts forward in explanation the concept of self and not self. According to this theory the scavenger cells of the body are able to distinguish between cells or cellular products which belong to host and material which is foreign. The latter is katabolised with antibody production and the former without. This theory postulates the existence of sub-cellular structures in the cells of the reticulo-endothelial system called recognition units. These correspond with a range of protein or polysaccharide self markers which confer the property of "self" on the body somatic tissue cells. When the appropriate recognition unit and self marker meet the material is set off on the path of digestion without antibody production. On the other hand antigenic material has a molecular pattern which differs from the body self markers and consequently react only partially with the body's recognition units. This partial

reaction leads to modification of the recognition units which are modified in such a way as to recognise and unite with the foreign material. These modified recognition units are in fact antibody molecules and the cells producing them transmit from one generation to another the capacity for their production. Antibodies have a fairly short life and accordingly the theory must postulate for long lasting immunity a perpetual growth and replication cells in which the modified recognition pattern is stored.

This capacity to distinguish between self and not self is not intrinsic and is "learnt" by the cells. Apparently the reticulo-endothelial system at an early stage in embryonic life accepts anything it meets with as "self" and if foreign substance is introduced at this stage the organism in adult life does not respond with antibody production to the particular material.

This principle of immunological tolerance has been ably expounded by Medawar and his colleagues (1953). These workers injected suspensions of liver and kidney cells from one group of mice (GPB) into the living embryo in utero of another group called here for convenience GPA. Some of these

mice were born alive and 4 - 6 weeks later were tested for their ability to accept skin grafts from Group B. The results were clear cut and showed that the mice treated in utero, unlike the normal controls, accepted the heterologous graft. If the mice are injected with heterologous cells during adult life they rapidly reject the foreign cells with an inflammatory reaction. There thus seems to be in the mouse some point before birth where foreign material is treated as self but later is rejected. The principle is substantiated also by Traub (1936, 1937, 1939) who developed a colony of mice saturated with L.C.M. virus. Infection took place in utero and the subsequently born mice failed to show antibodies although the virus was recoverable from all their organs.

Dunsford et al 1953 reported the interesting case of the woman blood donor with roughly equal amounts of O and A cells. The only reasonable explanation is that the woman had a twin and the two placental circulations had fused in foetal life. The foreign blood cells had been implanted at an early stage in development and were consequently recognised as self. It is recorded that when the donor was

asked if she was a twin she replied in surprise that she was.

There thus emerges an idea of 'self and not self' together with the principle of immunological tolerance and with this background it is now possible to briefly survey recent work on auto-immunity and the thyroid gland.

The concept of auto-immunisation came as a surprise to many because of the widespread belief that the body was unable to form antibodies against components of its own tissues. The first convincing evidence of auto-immunisation in man came from Roitt and Doniach (1957) who showed that a precipitation occurred when the serum of a patient with Hashimoto's disease was added to a saline extract of thyroid gland. They stated this was due to interaction between antigen from the gland and antibody in the serum. They believe that slow liberation of antigen from the thyroid gland results in the production of antibodies which progressively destroy the gland thus furnishing more antigen. In this way a chain reaction is brought about which may ultimately involve remote parts of the reticulo-endothelial system. Such a general immune response would then lead to further thyroid damage.

The Lancet (1957) points out that thyroglobulin dodges the principle of immunological tolerance since it is secluded within the acinus and is a stranger to the antibody forming cells.

Independently Witebsky and Rose (1956) demonstrated that a rabbit can produce antibodies against an extract of its own thyroid tissue. These antibodies are associated with constant and often severe damage to the animals' thyroid which has been left in situ. Professor Witebsky relates that a colleague Dr. Douglas Riggs first looked down the microscope at a damaged thyroid and made the significant remark "this looks like Hashimoto's thyroiditis." Since then these workers have demonstrated thyroid antibodies in human patients suffering from non-specific chronic thyroiditis.

These findings were confirmed by Anderson, Goudie et al (1957) who found frequent positive tests in patients with Hashimoto's disease and noted that a similar reaction occurred occasionally in thyrotoxicosis and primary myxoedèma.

It is of historical interest to remember that as long ago as 1936 Picado and Rotter published a paper with the auspicious title "Precipitins seriques anti-thyroidiennes chez les

goitreux" in which they mention high precipitin levels in myxoedema. Unfortunately much of their paper was obscure and some of their findings suggest they may have been misled by false precipitin reactions caused by unstable antigen. However in 1938 the same workers published another paper where they mention the possibility of "thyroid secretion working as antigen and calling forth the appearance of a serum precipitin." They also discussed whether thyroid antibody could produce an "alteration of function by virtue of a cytotoxic effect."

In 1958 (Lancet) Stuart, E.A. and Allan, W.S.A. described the occurrence of thyroid antibodies in carcinoma of thyroid and pointed out too much reliance should not yet be placed on serological procedures in the diagnosis of lymphadenoid goitre. This unexpected finding was confirmed by Doniach and Roitt (Lancet, 1958) who suggested that in such cases both lymphadenoid goitre and carcinoma co-existed.

Clearly a new concept in thyroid pathology has been born.

CHAPTER I.

(Section I.)

THE PATHOLOGY OF LYMPHADENOID GOITRE.

(Struma lymphomatosa or Hashimoto's Disease).

Introduction:

Following the first description by Hashimoto in 1912 struma lymphomatosa has been a source of controversy and even today there is still no general agreement on its essential pathological features. Since Joll's (1939) classical paper, new aspects of the disease have been uncovered and the purpose of this chapter is to review the pathological features in the light of this new knowledge. In 1953 Fromm noted the presence of raised serum gamma globulin levels and abnormal liver function tests in patients suffering from this disease. Later Roitt and Doniach (1956) related the raised gamma globulin levels to the presence of antibody to thyroglobulin and suggested that these antibodies progressively destroyed the gland. Recent advances in histological technique (Slidders and Lendrum, 1958) have made the study of basement membrane damage in the thyroid gland a relatively simple matter and the aim of part of this thesis is to demonstrate and discuss such changes.

In view of the lack of unanimity on the

histological features of lymphadenoid goitre it is helpful to review briefly the changes seen by Hashimoto. He described four cases of unusual goitre in elderly females. The glands all showed diffuse parenchymal changes, lymphoid infiltration and fibrosis.

The parenchymal changes consisted of diminution in size of the acini which were lined by a single layer of epithelium, sometimes infiltrated by mononuclear leucocytes. In case 1 the follicles were generally large, in cases 2 and 3 considerably reduced in size and in case 4 they were markedly atrophic. "Especially in case 4 the smaller vesicles predominate. Here there are also such areas that consist almost entirely of very small vesicles about 30 μ diameter, indeed often even epithelial cells lying side by side haphazardly in heaps. Thus one can no longer recognise any indication of vesicle structure."

The lymphoid infiltration was widespread and showed lymphoid follicles with germinal centres and diffuse interstitial infiltration by lymphocytes and small numbers of plasma cells. In case 1 the round cell infiltration was slight, in cases 2 and 3 moderate and in case 4 strikingly

pronounced. The round cells consisted of lymphocytes and plasma cells whose "protoplasm and nuclei were typically stained." The description of moderate numbers of plasma cells by the original author is important because some workers (Terplan, 1957) are reluctant to diagnose "Hashimoto's disease" where such cells are present.

Fibrous tissue proliferation was present in all Hashimoto's cases. "It was in marked proliferation everywhere, which gave a beautiful picture with van Gieson's stain. It was diffuse and especially evident in case 4, so that one could not recognise the lobular arrangement of the vesicles."

This summary emphasises that Hashimoto considered his cases showed a progression of pathological changes, that fibrosis could be marked and that plasma cells were present.

In 1923 Simmonds described the pathology of 20 cases of chronic thyroiditis which he believed would progress by fibrosis to complete atrophy. Although he made no mention of Hashimoto's paper it seems probable that some of his patients resembled Hashimoto's case 4.

In 1925 Williamson and Pearse introduced the term "lymphadenoid goitre" which they defined by lymphocytic aggregations, fibrosis and a peculiar atrophy of the parenchyma. To them it was a specific progressive condition, which in the early stages showed much round cell infiltration, the latter eventually replaced by connective tissue with consequent fibrosis and myxoedema. They pointed out that it could sometimes be focal and not goitrous. Their paper makes no mention of either Hashimoto or Riedel.

In 1937 Vaux described the pathology of 38 cases of lymphadenoid goitre and divided them into early, intermediate and late stages depending on the degree of parenchymal atrophy and fibrosis. In her opinion there was a definite gradation in the degree of parenchymatous destruction and the amount of fibrosis, the average age of the patients corresponding with what might be expected in the three gradations.

In 1946 Parmley and Hellwig emphasised the striking epithelial changes which occur in lymphadenoid goitre. Each of their 14 cases showed large pale oxyphilic cells which they likened to hepatic or adrenal cells. They were

among the first to point out the distinctive thyroid epithelial change - Askanazy or Hürtle cell change - in this condition and suggested that this might represent the primary alteration. The more conspicuous lymphoid infiltration would then occur as a secondary phenomenon.

There thus arises a dynamic concept of lymphadenoid goitre in which the excess of lymphoid tissue is replaced by connective tissue with final fibrosis and complete atrophy of the epithelial elements.

This report is based on the personal examination of 100 biopsy and thyroidectomy specimens from the files of the Royal College of Physicians Laboratory and Pathology Department of the Edinburgh Royal Infirmary. Where possible the case notes were studied and no case is included where there is a history of severe pressure, symptoms or dense periglandular adhesions. The histological diagnosis is based on the simultaneous presence of a triad of features, viz.

1. diffuse Askanazy change of the thyroid epithelium.
2. lymphoid infiltration with or without germinal follicles.
3. a variable degree of fibrosis.

These three essential abnormalities are present in all glands studied but differ in quantity in each specimen. When lymphoid and epithelial elements predominate the gland is classified as a lympho-epithelial phase. If there is a predominance of lymphoid and fibrous elements the term lympho-fibroid phase is used. In the last phase the lymphoid tissue is almost completely replaced by connective tissue and the term fibrous phase is applied. (Table 1).

Table 1.

Classification of 100 cases into three phases.

Lympho-epithelial	78
Lympho-fibrous	17
Fibrous	5

The essential histological features of these phases will now be described.

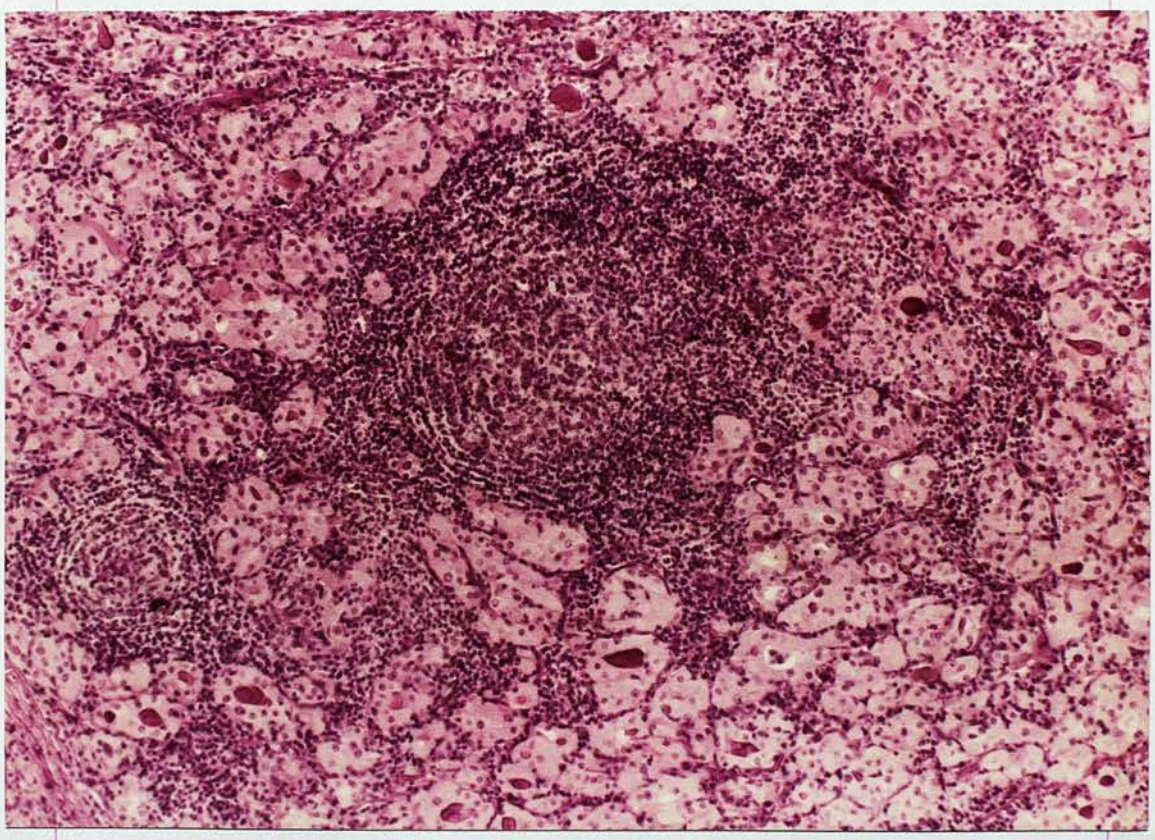


Fig. 1. (H.E. x 120.)

Classical appearance of lymphadenoid goitre showing prominent lymphoid follicles, small acini and moderate degree of interstitial round cell infiltration.

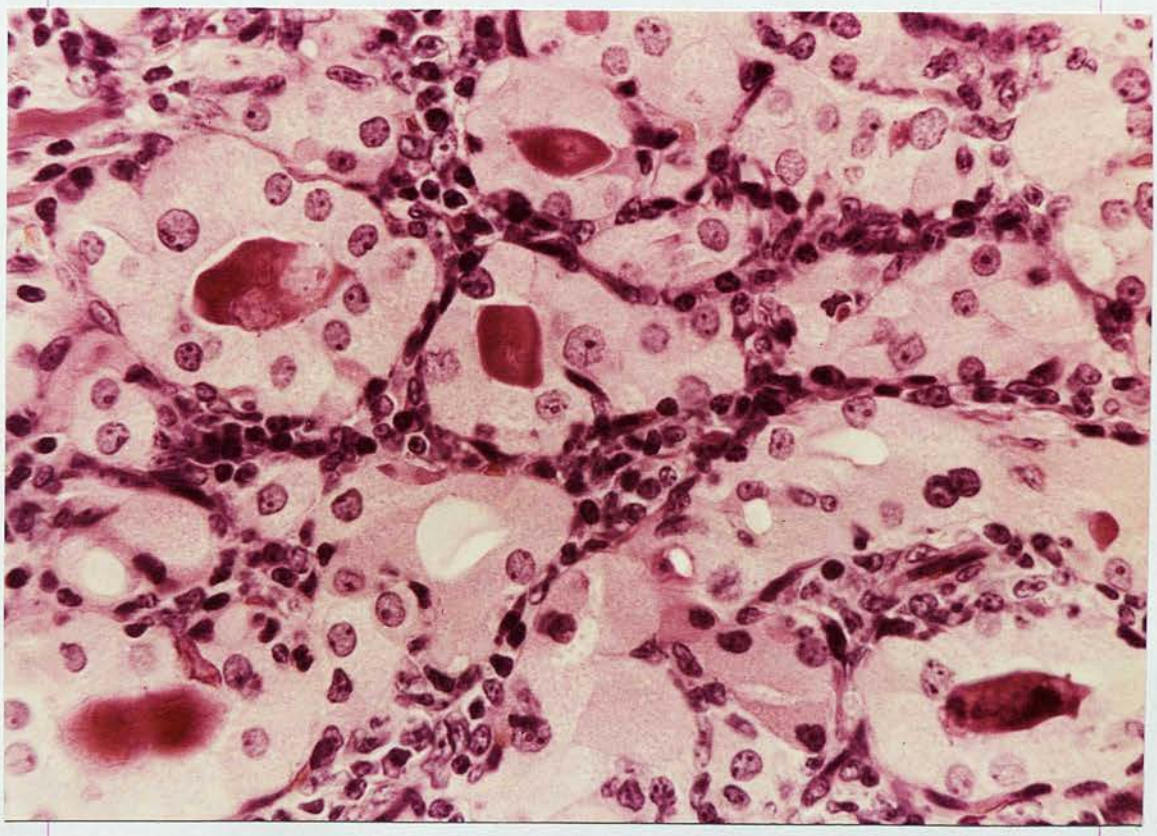


Fig. 2. (H.E. x 150).

Characteristic epithelial cell changes.
The epithelium is plump and cuboidal with faintly granular oxyphilic cytoplasm. The nuclei vary in position and some acini contain fewer nuclei than usual. This appearance is sometimes called Askanazy, Hürtle or pink cell change. Small foci of Askanazy cell change are sometimes seen in thyrotoxicosis and in such instances the oxyphilia and nuclear pleomorphism is usually more marked than in lymphadenoid goitre.

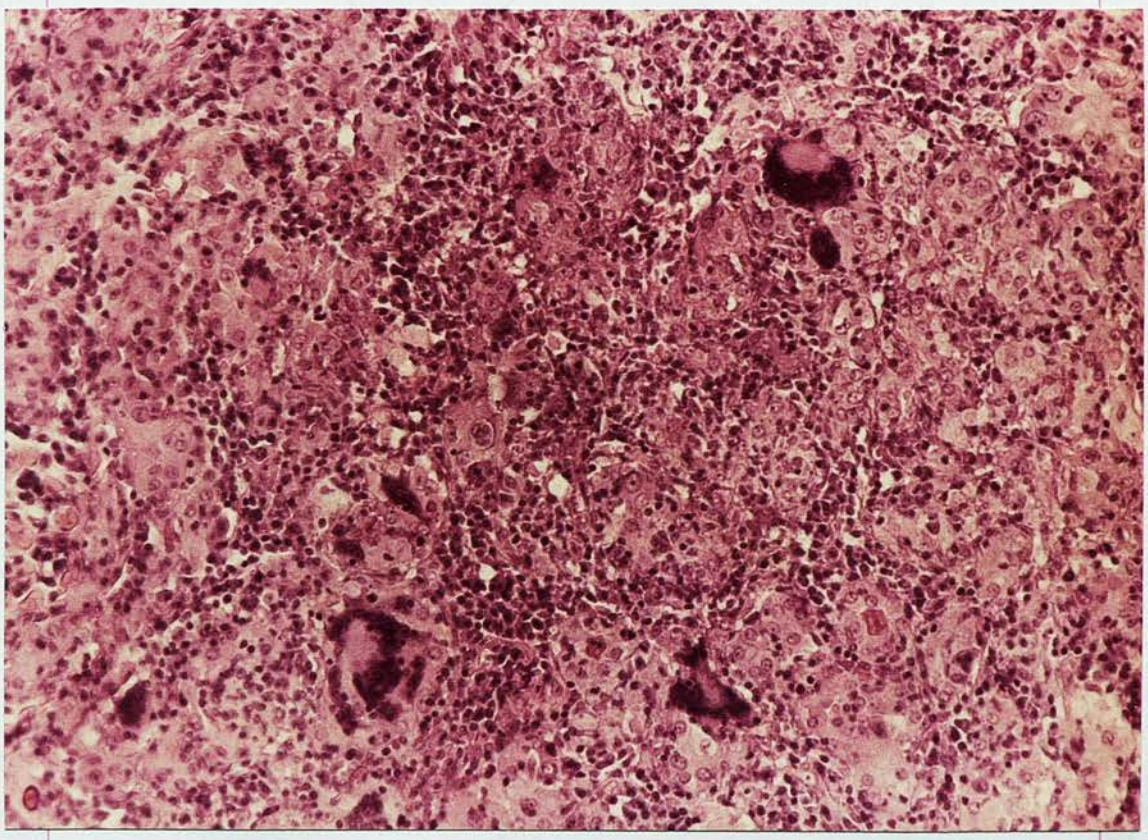


Fig. 3. (H.E. x 200).

Giant cells in Hashimoto's disease. These are found in approximately 10% of cases and when numerous the condition may be mistaken for De Quervain's thyroiditis. The writer agrees with Goetsch (1940) that these cells are derived from thyroid epithelium and are not true foreign body giant cells.

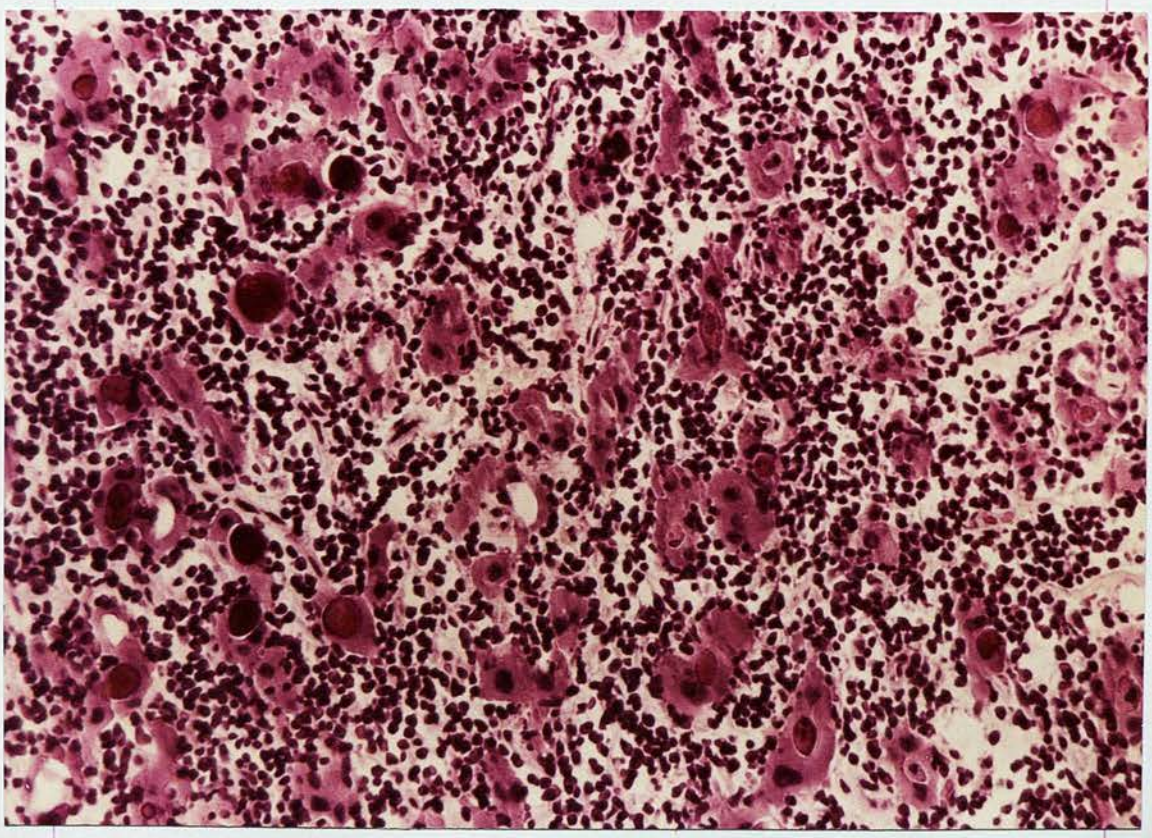


Fig. 4. (H.E. x 240).

Diffuse lympho-epithelial type. The parenchyma is broken up into groups of small acini showing Askanazy cell change and diffuse round cell infiltration. The essential histological features are all present and the picture differs only quantitatively from the classical appearances of Fig. 1. A similar section is illustrated by Renton, Charteris and Heggie (1938) whose otherwise exemplary paper calls the condition "Riedel's disease."

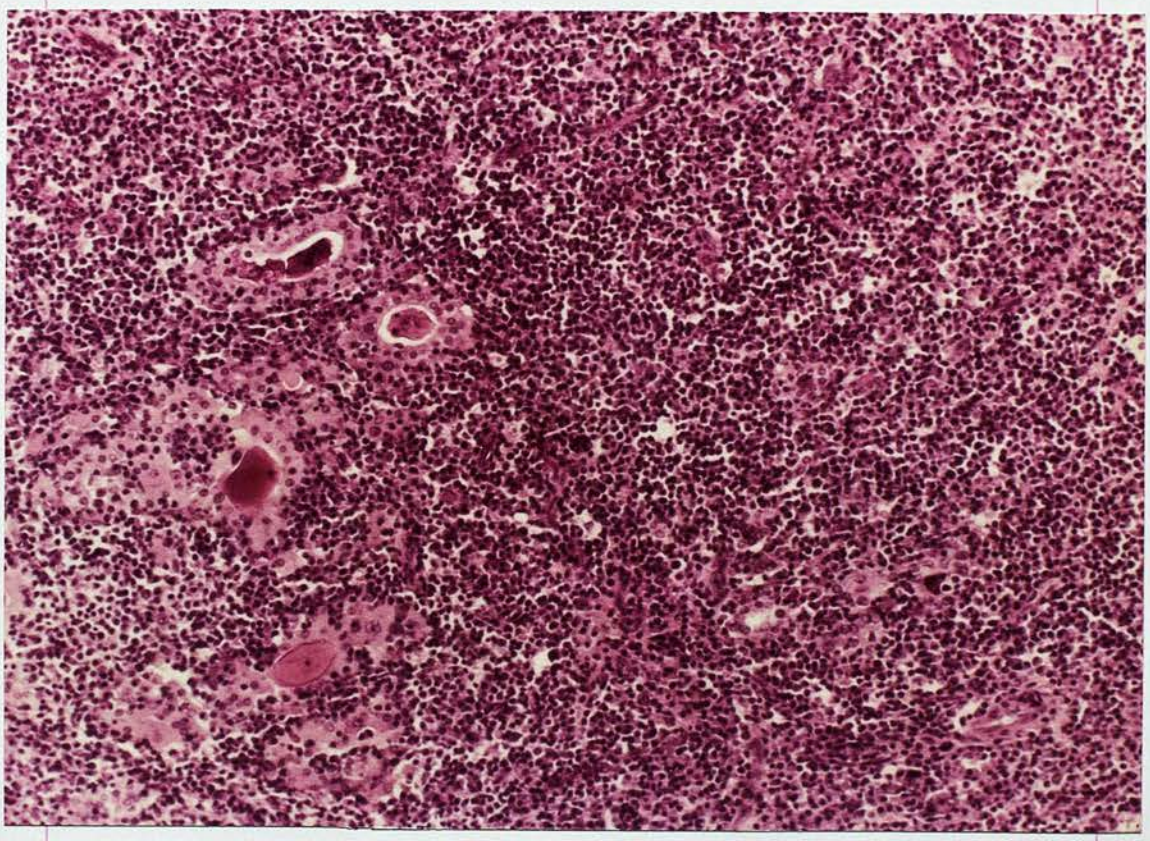


Fig. 5. (H.E. x 140).

Dense round cell infiltration. Occasionally this appearance may be confused with small round cell carcinoma and Kellett (1937) mentions instances where this mistake has been made. A higher magnification (fig. 6) however shows numerous plasma cells.

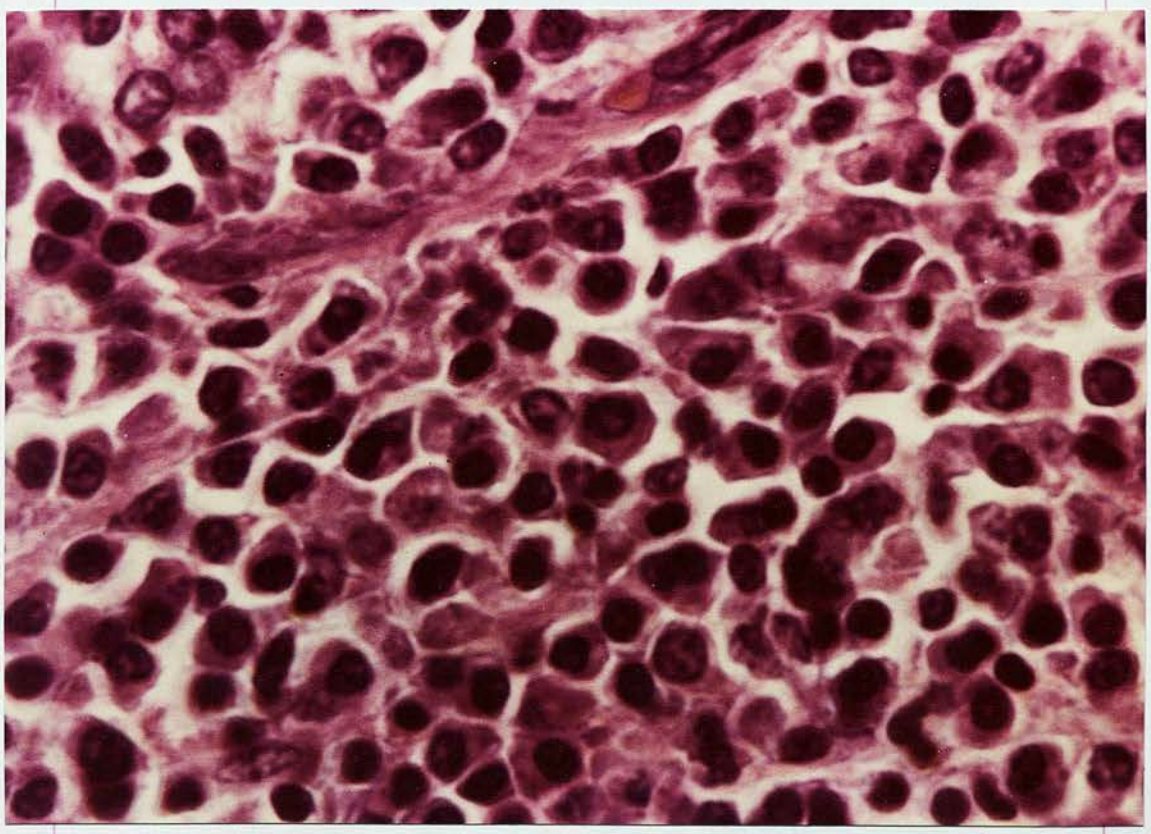


Fig. 6. (H.E. x 1200).

Numerous plasma cells. Such a dense plasma cell infiltration is unusual. Only two "plasmacytomas" of the thyroid were found in the present series.

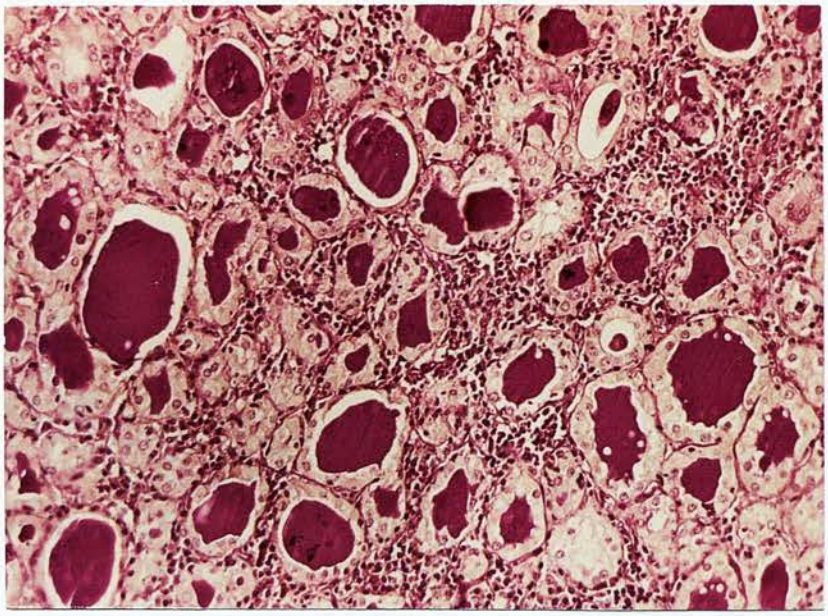


Fig. 7.

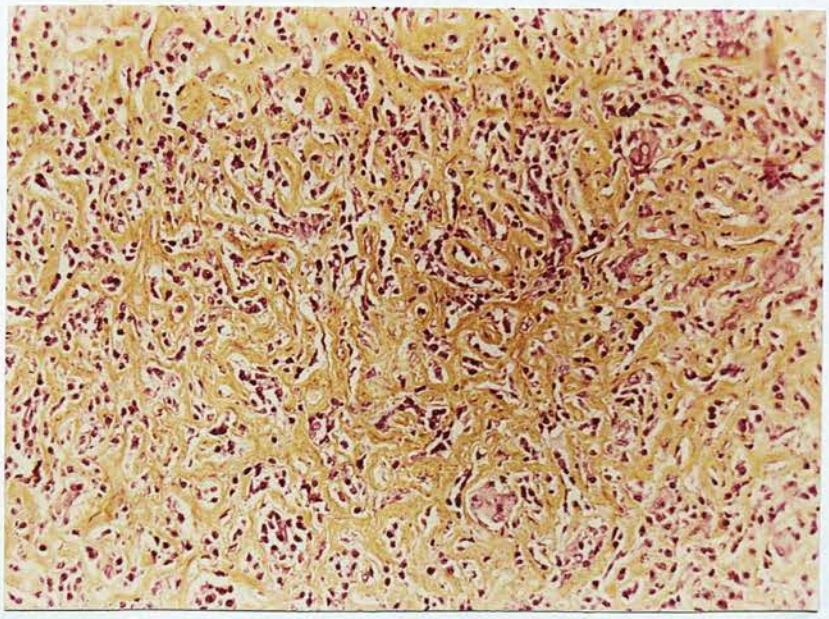


Fig. 8.

Figs. 7 and 8.

These illustrate the marked variations in histological appearances which may occur in the same gland. Both photographs were taken from the same section. Fig. 7 shows the usual lymphadenoid appearances and Fig. 8, (Fuchsin-Lissamine yellow x 150), reveals marked fibrosis - yellow areas.

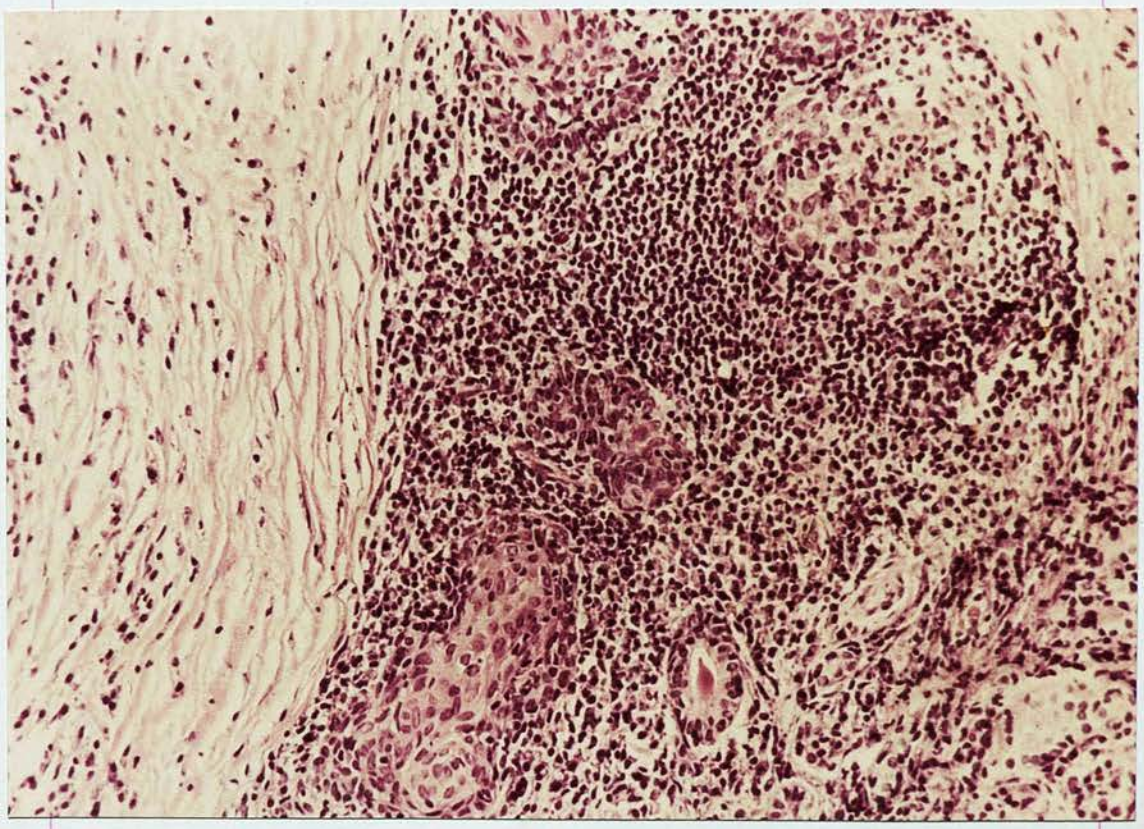


Fig. 9. (H.E. x 200).

Squamous metaplasia. This is commonly found in the lymphofibrous phase. It has been beautifully illustrated by L.H. Meeker (1925) who preferred however to regard the nests of squamous cells as remnants of post branchial body.

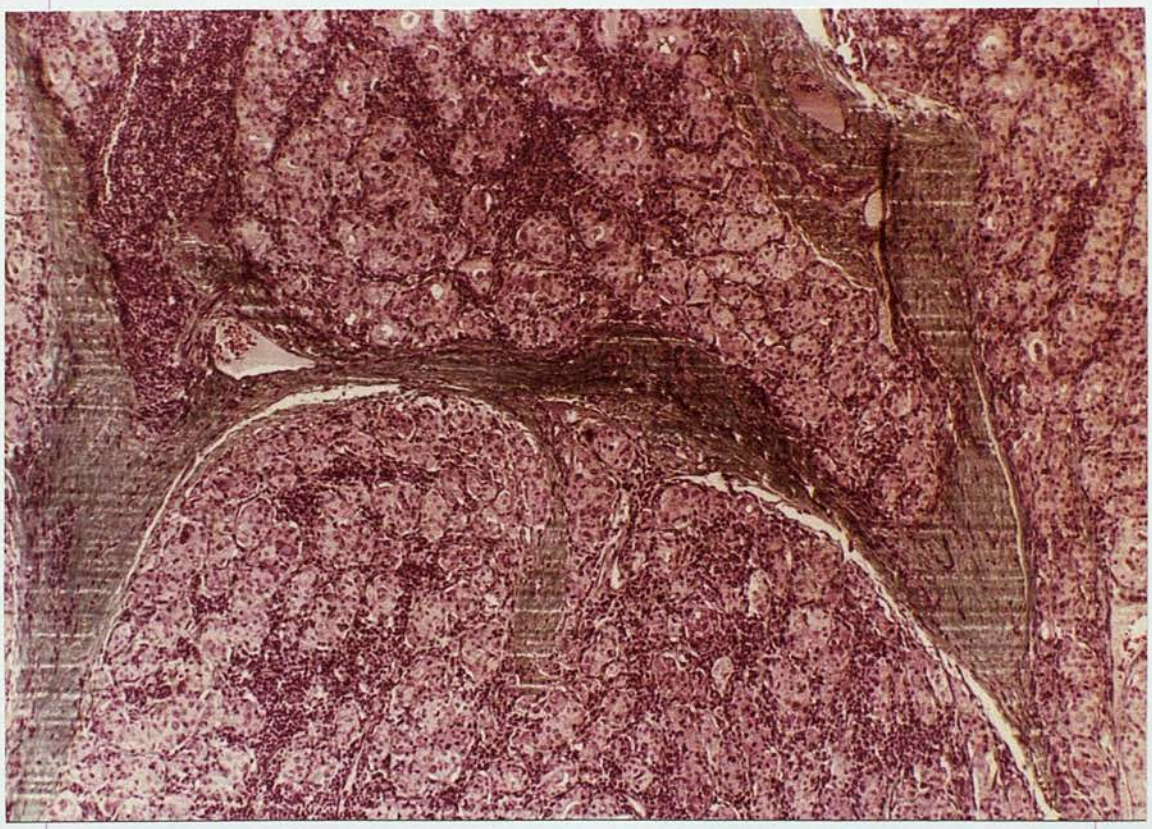


Fig. 10. (Masson trichrome x 90.)

Lympho-fibrous phase (early). This shows early lobulation and moderately thick fibrous tissue trabeculae.

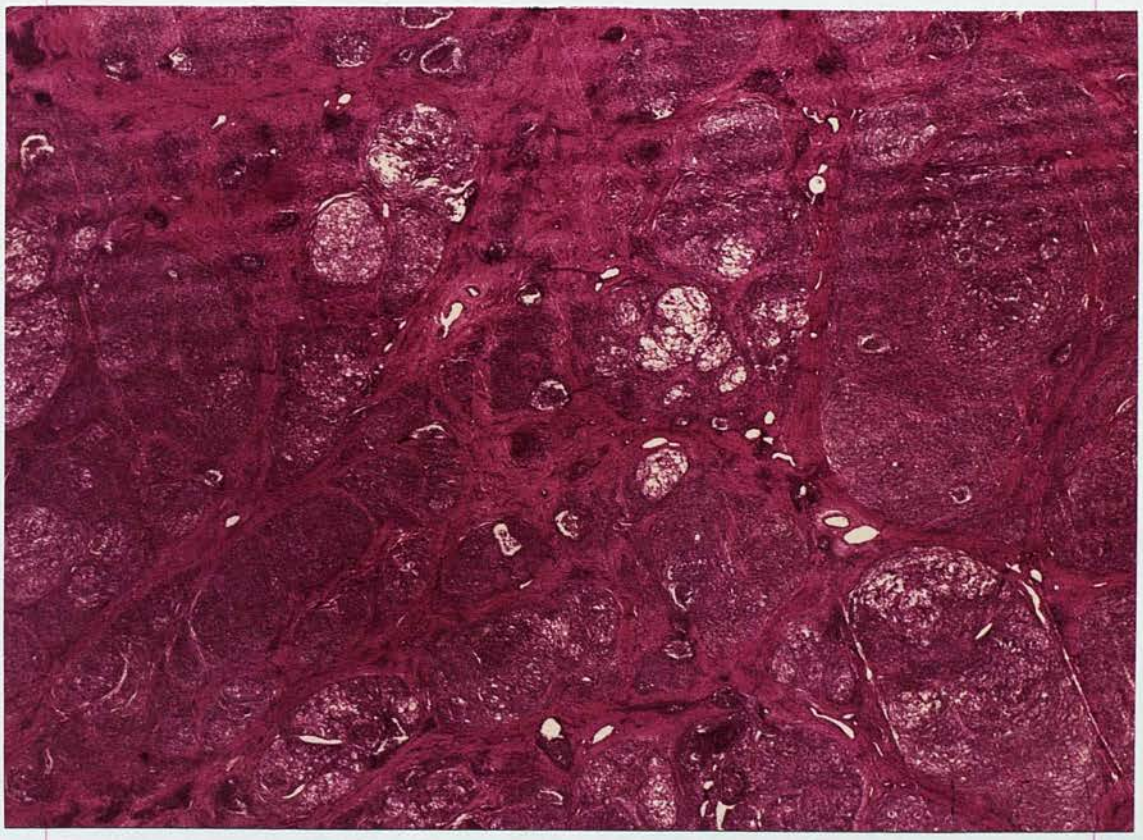


Fig. 11. (H.E. x 8).

Lympho-fibrous phase. Lobulation is marked and the parenchyma is divided by interlacing bands of dense fibrous tissue. Fibrosis in this condition proceeds by way of replacement of lymphoid tissue and thus differs from the fibrosis of subacute thyroiditis which resembles scar formation.

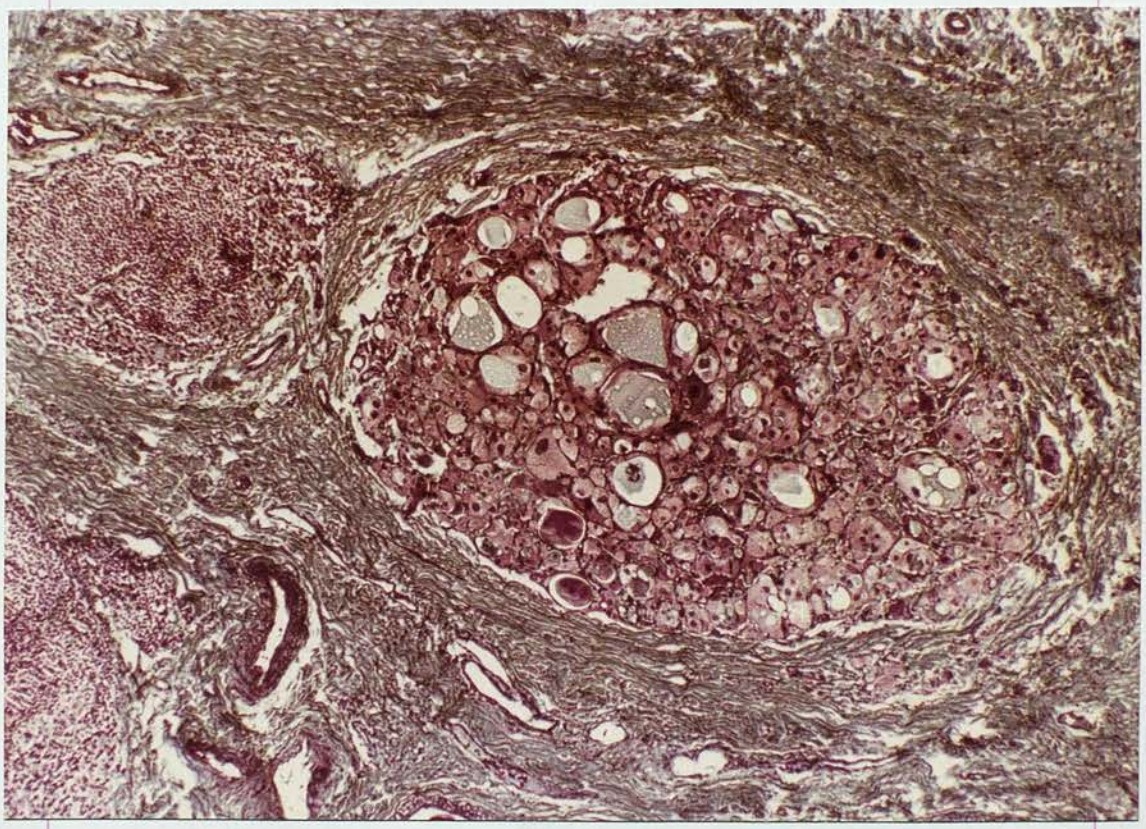


Fig. 12. (Masson trichrome x 90).

Lympho-fibrous phase. The fibrosis is more advanced than fig. 11. The follicles are small and the epithelium still retains the distinctive Askanazy cell change. Two lymphoid follicles are also present. The appearances are not unlike those seen in cirrhosis of the liver.

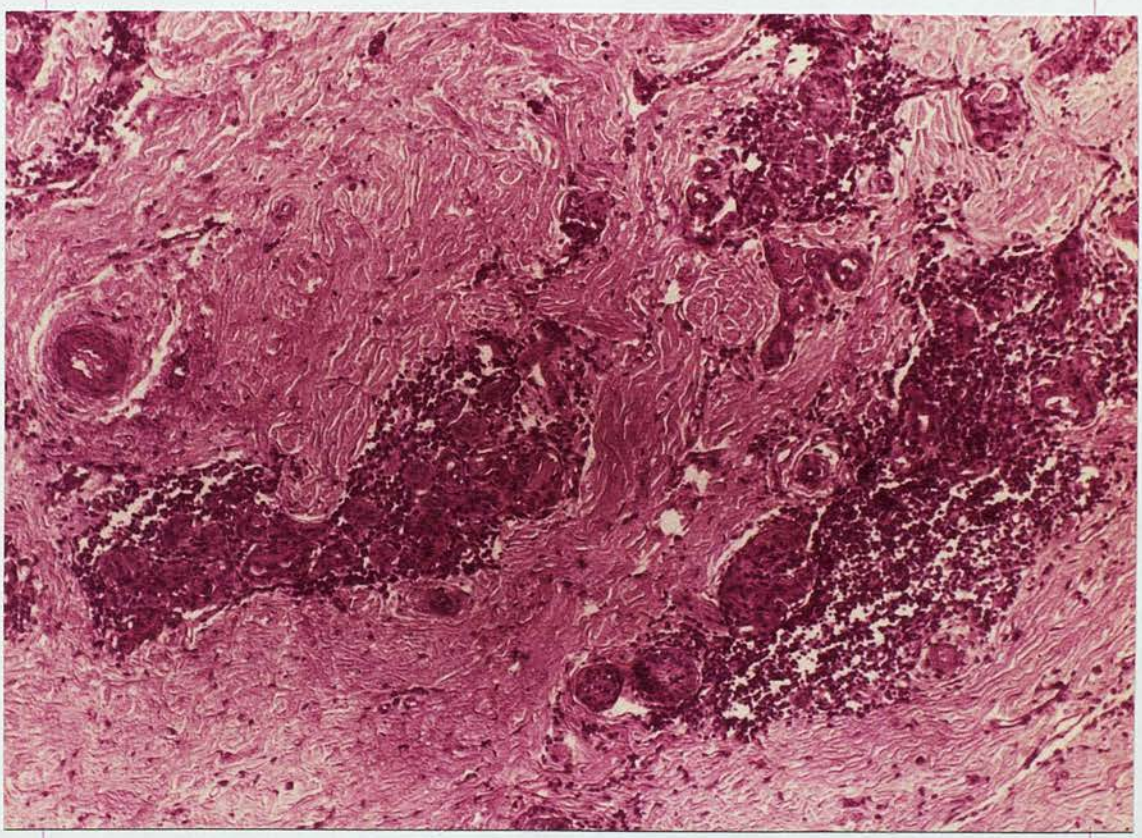


Fig. 13. (H.E. x 90).

Fibrous phase. Massive fibrosis enclosing a few small islands of thyroid parenchyma which shows a diffuse round cell infiltration and striking Askanazy cell change.

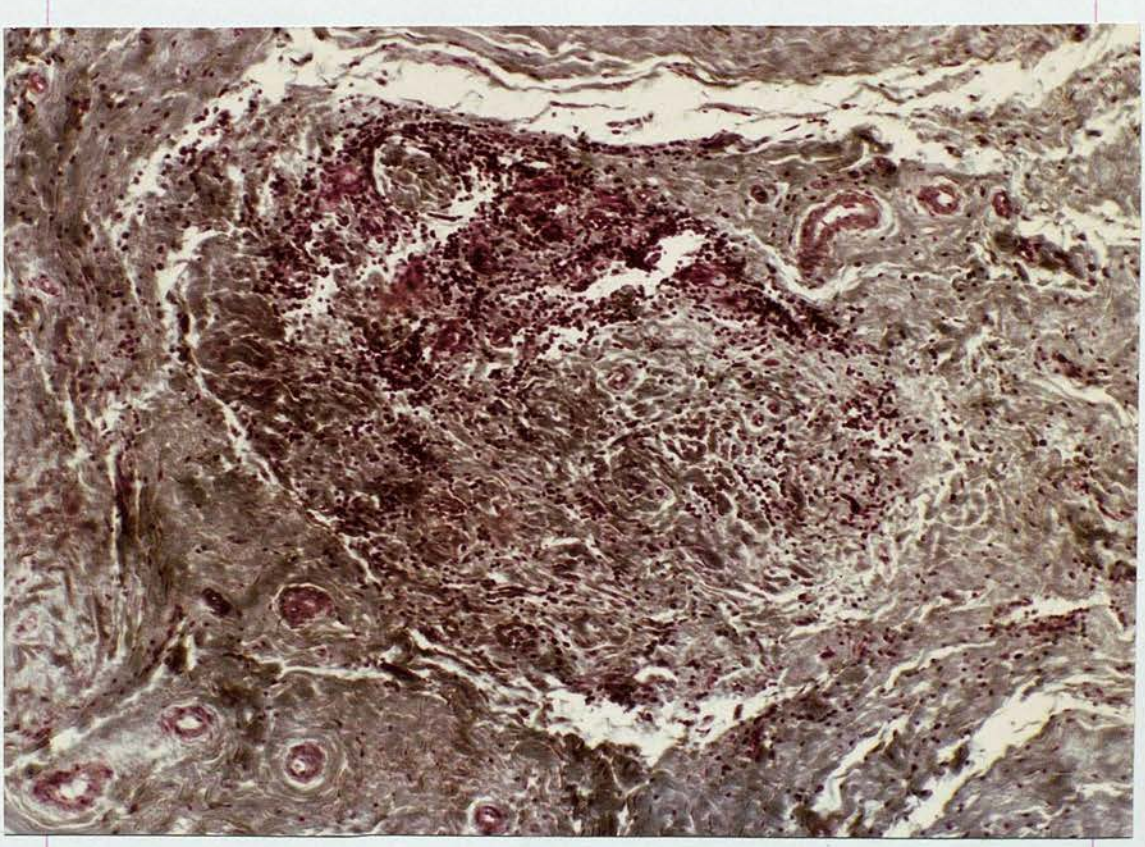


Fig. 14. (Masson trichrome x 130).
Fibrous phase. Complete fibrosis.

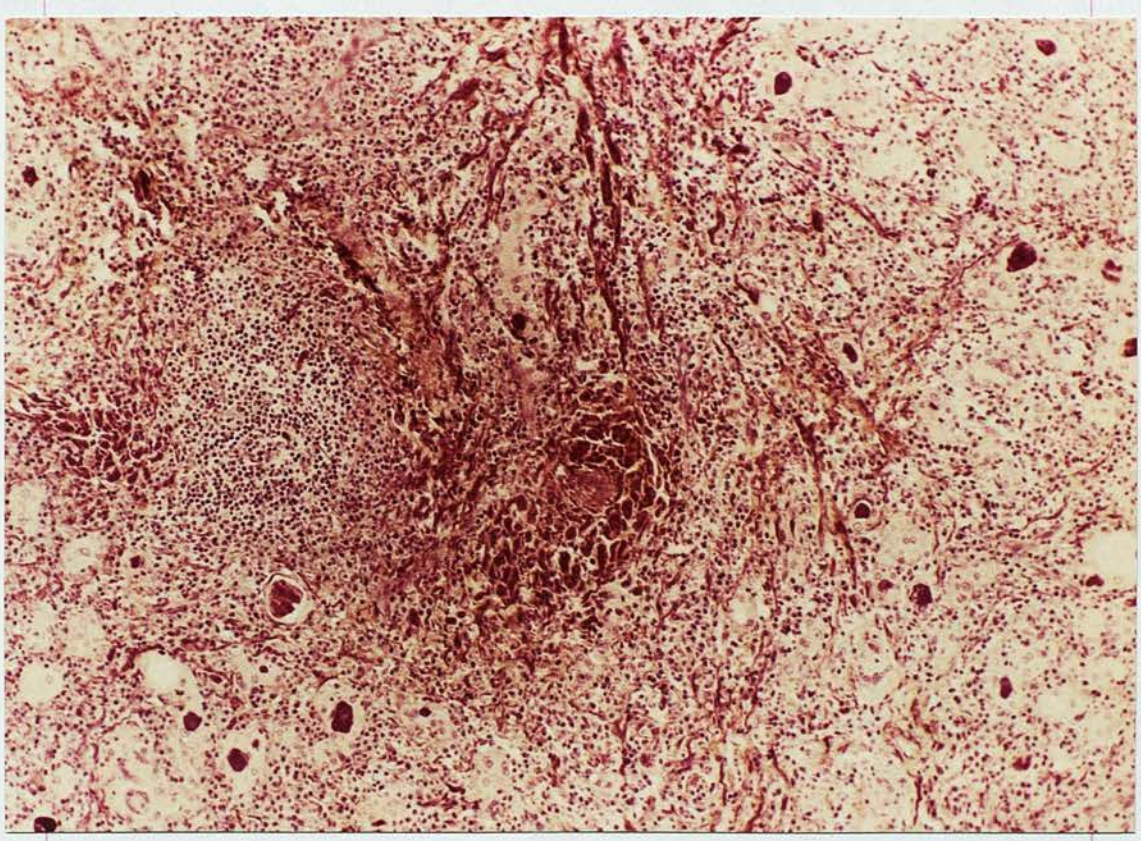


Fig. 15.

"Hyaline" Trabeculae: Fuchsin-Lissamine Fast Yellow x 130.

In sections stained with H.E. the fibrous tissue bands have a homogeneous appearance. Connective tissue stains such as Gomori trichrome or the acid picro-Mallory show that many of these fibres fail to stain for collagen because they are strongly fuchsinophilic and in consequence appear bright red.

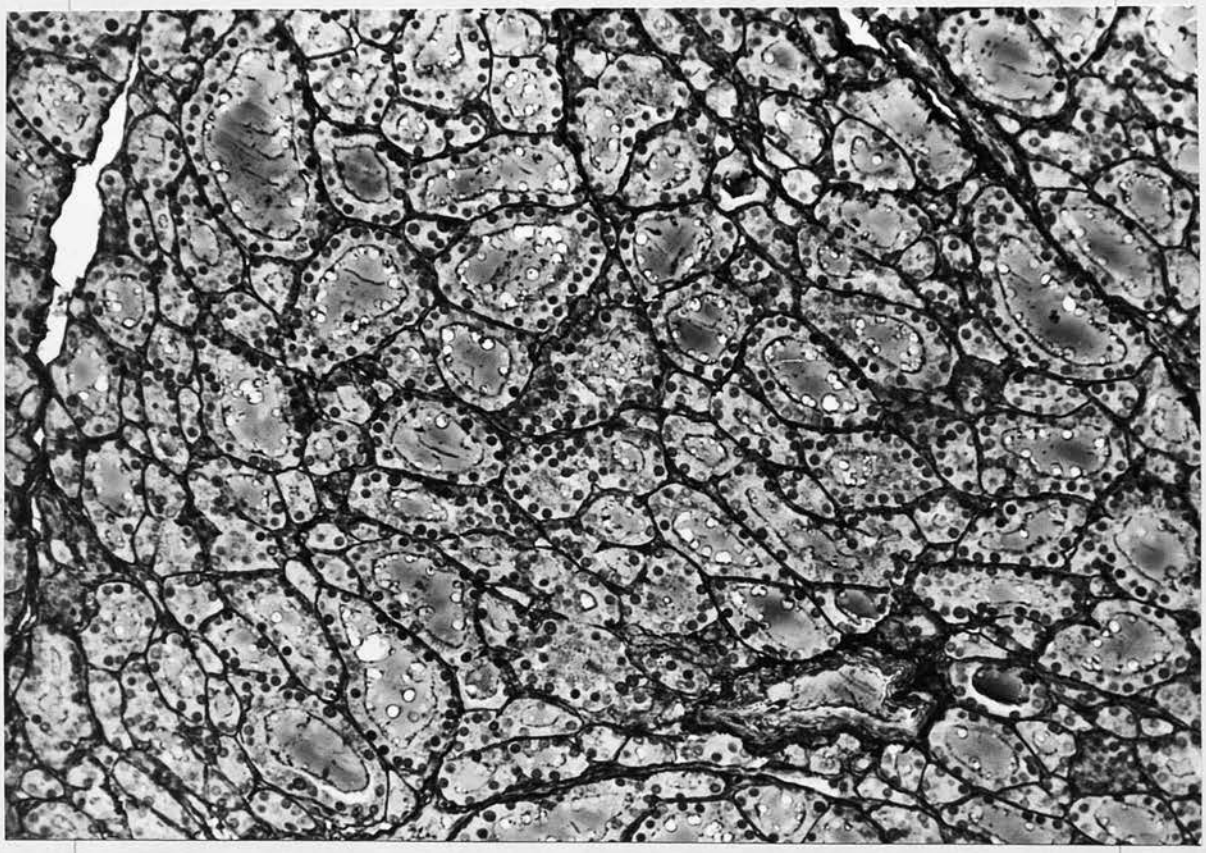


Fig. 16.

Normal thyroid: (Silver impregnation x 210).

The basement membrane in a normal gland stains intensely and evenly; it measures approximately 0.1 to 0.2 μ in width and is closely applied to the base of the epithelial cells. It forms a continuous unbroken lining and completely seals off the follicle from capillary vessels.

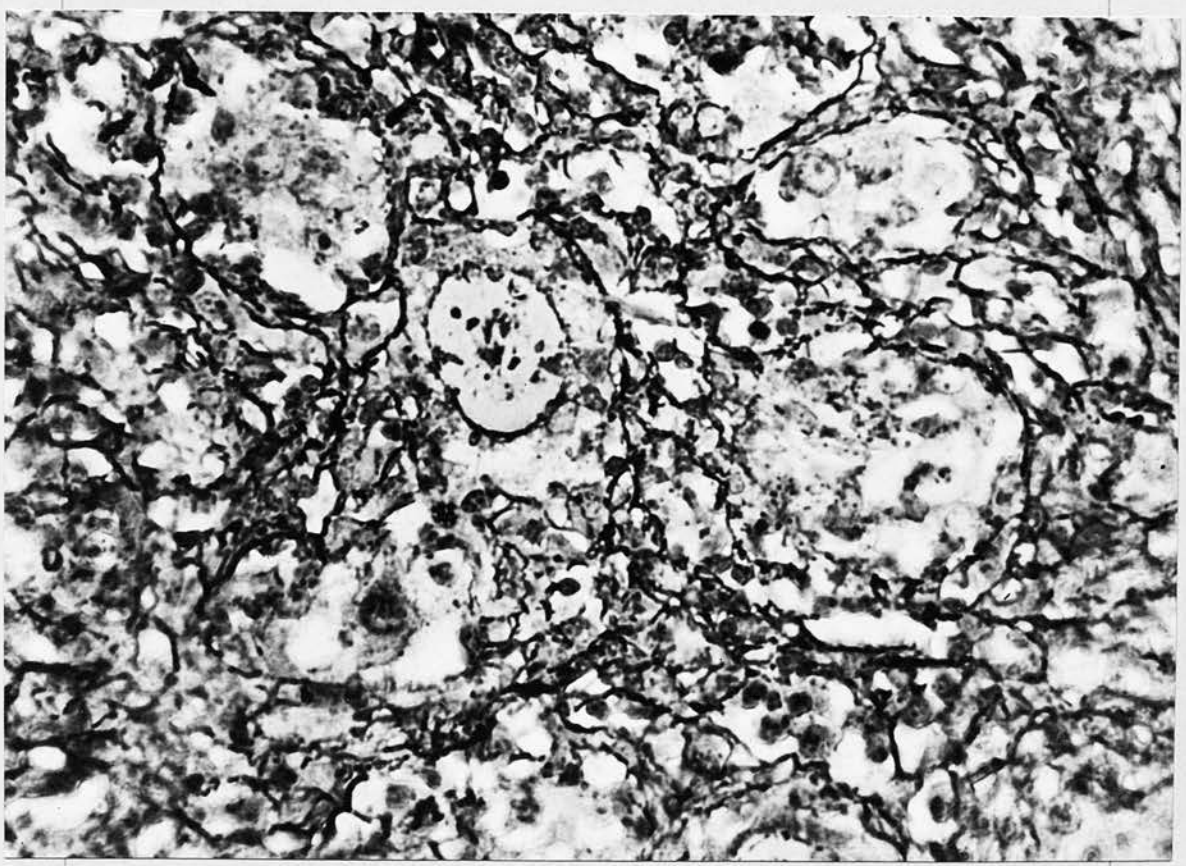


Fig. 17.

Lymphadenoid goitre (Silver impregnation x 600)

The basement membrane is irregular, wavy and fragmented.

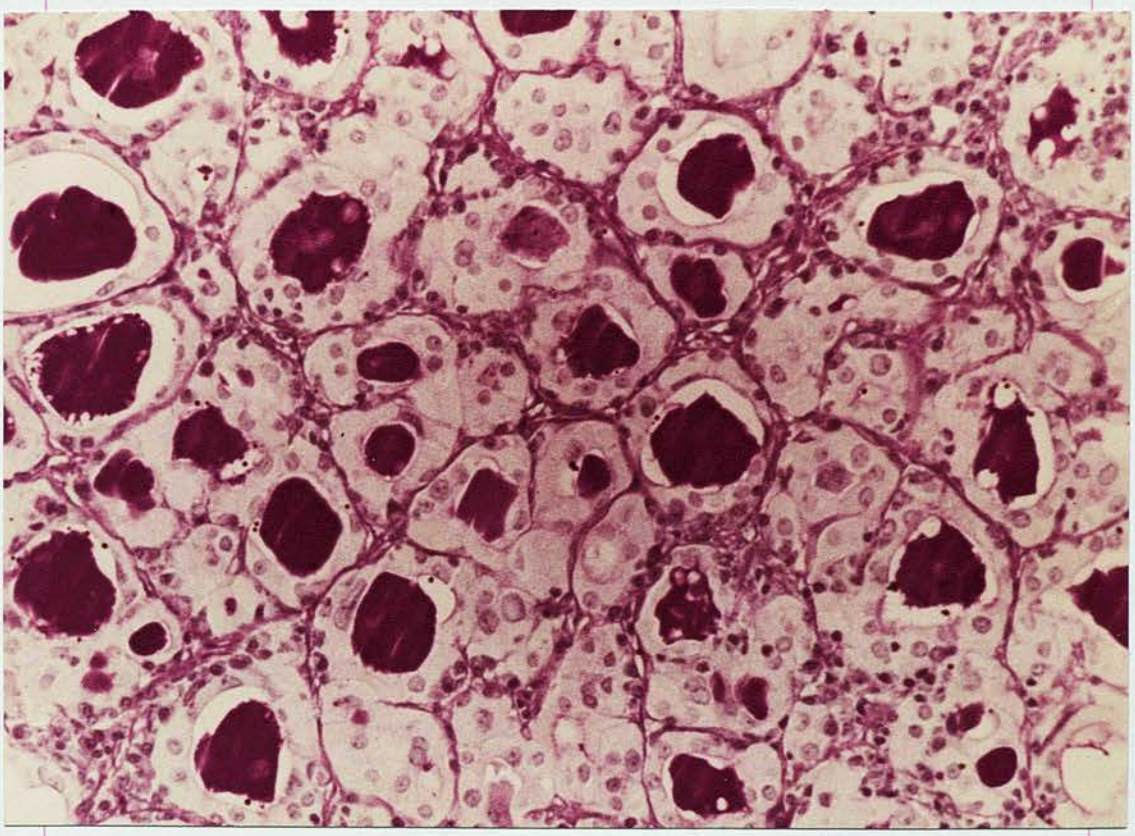


Fig. 18.

Lymphadenoid goitre (P.A.S. x 280).

The basement membrane is purple-red. It is irregular, lacks continuity and in some places is diffusely thickened.

Fig. 19.

Germinal centre of lymphoid follicle in Struma Lymphomatosa - Fuchsin-flavine x 90. (author's own stain, see appendix).

Fuchsinophilic material is commonly seen within the lymphoid tissue.

See two pages over.

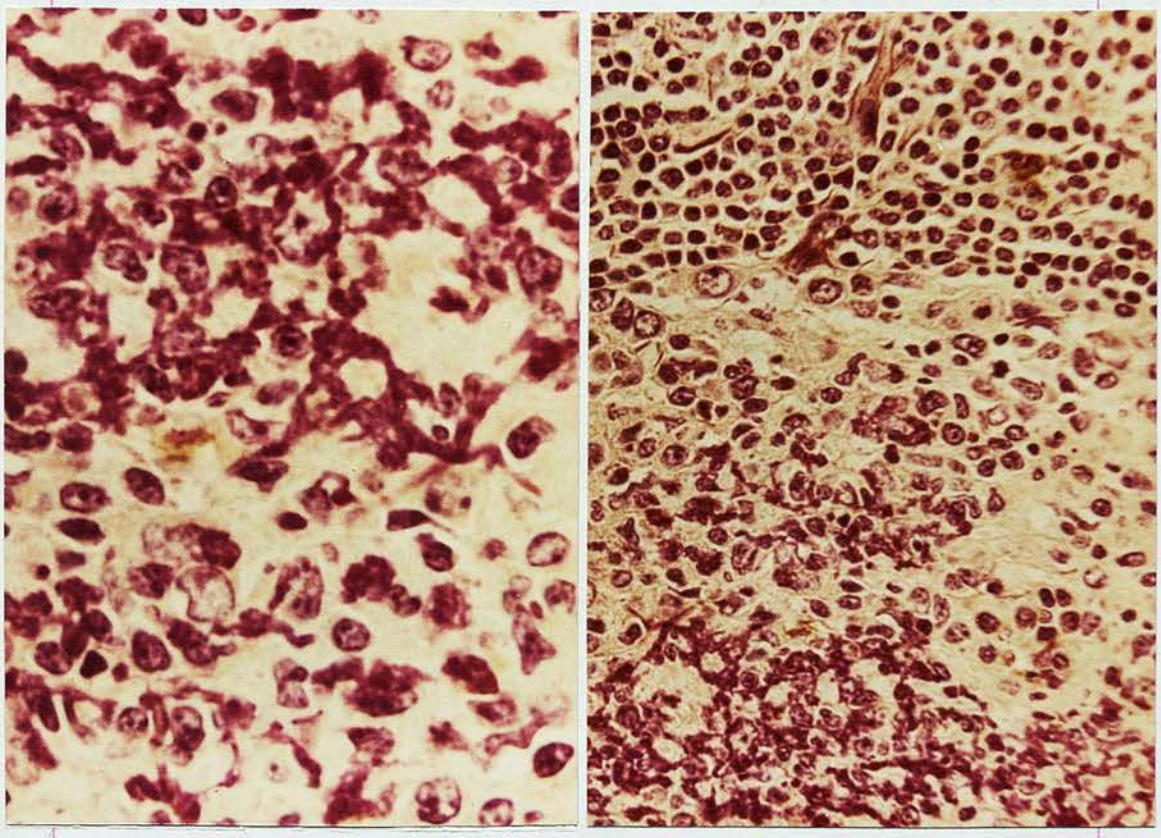


Fig. 20.

Same field and stain as fig. 19 x 1000 & 550

This shows the fine structure of the fuchsinophilic material most of which lies between cells but some is intra cellular.

See one page over.

Figs. 19 and 20.

Morphology of Fuchsinophilic Material.

This material is seen usually at the periphery of the germinal centre of the lymphoid follicle in Hashimoto's disease although sometimes it is present in the central parts. It consists of small granules and rods which have a dense homogeneous appearance. The granules are round and measre 1 to 2 μ in diameter. Occasionally they are larger and resemble small erythrocytes. The rods measure 1 μ in thickness and 2 to 3 μ in length. Frequently the material forms small irregular heaps, distributed unevenly through the follicle. This is the characteristic appearance when the material is scanty and a rather different picture is seen when large amounts are present. In such instances two forms are seen. Firstly a branching twig like appearance composed of agglutinated rods and granules. Secondly small irregularly shaped lakes of dense homogeneous material. Under the oil immersion lens sinus-like spaces bordered only by reticulum cells can be seen and this material is frequently seen at the edges of these spaces. These spaces admittedly may be an artefact caused by the routine prefixation of paraffin sections and accordingly one cannot believe that they are true sinuses.

Figs. 19 and 20 (Contd).

Most commonly the material lies between cells but sometimes it can clearly be seen within the cytoplasm of large mononuclear cells. It is restricted entirely to the germinal centre and is rarely, if ever, present in the surrounding zone of lymphocytes.

Stain reactions.

Stains	Results.
Fuchsin fast yellow	bright red
P.A.S.	negative or faint
Feulgen	negative
Weigert's fibrin stain	negative
P.T.A.H.	positive
acid Picro-Mallory (Lendrum)	red
Masson trichrome	pinkish-red, not well defined.
Amyloid (methyl violet)	negative.

Discussion:

The difficulty in classifying cases of Struma lymphomatosa is apparent to anyone afforded the opportunity of examining a large series. Not only do the pathological changes vary from gland to gland but even within the same gland marked variations occur. When only a solitary biopsy specimen is available it is indeed hazardous to assume the changes are representative but since most of our material consists of thyroidectomy specimens it was possible to form a rough estimate of the relative amount of fibrous tissue and classify them accordingly. This study unreservedly supports the views of Simmonds (1923), Williamson and Pearse (1925) and Vaux (1937) all of whom advanced the concept of struma lymphomatosa being a progressive disease leading to fibrosis and myxoedema. The evidence obtained from repeat biopsy at intervals of months or years is difficult to evaluate. McClintock and Wright (1937) recorded identical clinical and pathological findings in a case of struma lymphomatosa investigated on two occasions at an interval of two years. Furr and Crile (1954) have even recorded no change after an interval of 21 years. On the other hand Heyd (1929) found to the

contrary and recorded increased fibrosis and diminished round cell infiltration on the second occasion. In the present series one patient had first one lobe removed and then the other two years later. The microscopic findings were the same in either case. A second patient had a repeat biopsy after an interval of approximately eighteen months when a slight to moderate increase in fibrous tissue was noted. In view of the singular lack of uniformity of histological appearances in struma lymphomatosa one is not prepared to draw any conclusions from this isolated finding. The natural history of struma lymphomatosa is still largely a mystery but at least it seems certain that the rate of progression of the pathological process may vary considerably.

The acceptance of a lymphofibrous phase in struma lymphomatosa is quite in accord with Hashimoto's original description,and indeed his fourth case appears to belong to this group. Unfortunately the lymphofibrous phase has been widely interpreted as Riedel's illness and this has been partly responsible for the sterile controversy of whether Hashimoto's disease was an early stage of Riedel's struma. When fibrosis is very marked it is of course impossible by

histological examination to discover the nature of the antecedent pathology since several quite different processes such as Riedel's illness, subacute thyroiditis or irradiation, may lead to thyroid fibrosis. It must be emphasised that the fibrous glands illustrated in this section were in fact goitres and their large size distinguishes them from the small fibrous gland frequently found in myxoedema. It seems probable that shrinkage and fibrosis within a lymphadenoid goitre may eventually lead to the small gland seen at necropsy of patients with myxoedema, but one can offer no proof on histological grounds alone that this occurs.

The basement membrane changes in lymphadenoid goitre are striking, severe and constant. Such changes were found in all of twenty thyroids which were stained specially for this purpose. The method used to demonstrate the basement membrane is of importance. The P.A.S. method lacks clarity and crispness and of all the methods tried the most suitable was the modified silver stain of Slidders and Lendrum (1958). Fragmentation of the basement membrane is nearly always associated with the presence of chronic inflammatory cells in the affected part. In some instances damage to

the membrane was seen in the absence of a leucocytic infiltrate and accordingly it seems probable that this is an early change which permits the escape of colloid into the interstitial tissue. Colloid is a tissue irritant (Ferguson, 1937) and the consequent inflammatory reaction would then explain the frequent co-existence of a pathological basement membrane with lymphocytes and plasma cells.

The author believes that the basement membrane pathology is an integral and fundamental part of the histo-pathology of struma lymphomatosa and the significance of this change is described later in detail. (Chapter VI : The Significance of Basement Membrane Change in Thyroid Disease).

Askanazy cell change: All cases reported here showed diffuse 'pink cell' change and material in which this abnormality was absent has been rejected as unsuitable for inclusion in the study. Goitres showing all the features of struma lymphomatosa with the exception of Askanazy change are uncommon and only four cases have been encountered by the author. To impose such a restricted diagnostic criterion may appear injudicious but in so doing one is able to preserve

the essence of Hashimoto's original description and exclude other disorders such as the lymphogenous hyperplasia of toxic goitre and Crile's "lymphoid thyroiditis" (1953). In both of these conditions the lymphoid infiltration may closely resemble that of Struma lymphomatosa but in neither is diffuse Askanazy change observed. This change is so frequent and so striking a feature of Struma lymphomatosa that one cannot but believe it forms an integral part of the disease.

Askanazy cell change in our material was nearly always associated with plasma cell infiltration but on several occasions it was seen on its own which suggests that it precedes the inflammatory cell infiltration. The nature of the Askanazy change is unknown. Its existence was recognised by Hashimoto but scanty attention was paid to it until Parmley and Hellwig (1948) drew attention to the distinctive nature of the epithelial cell change and suggested that this and not the more conspicuous lymphoid infiltration might represent the primary and fundamental alterations. The question arises as to whether this alteration is a result of immunological

injury but this does not seem likely since thyroid cells in tissue culture fail to show a similar degenerative change when exposed to Hashimoto serum (personal observation).

The lymphoid fuchsinophilic material: This curious material has been noted within the germinal centres of the lymphoid follicles seen in Hashimoto's diseases, where it was observed in eight out of ten cases. Although this material is very frequently seen in Hashimoto's disease it has been occasionally observed in chronically inflamed lymph nodes. The appearances are striking and although many pathologists must be familiar with it the material does not seem to have been described in the literature. It is seen with difficulty in sections stained with haematoxylin and is best demonstrated by the acid fuchsin method described later in the appendix.

The material is not red cells since the acid picro-Mallory stains the erythrocytes bright yellow. It is not likely to be collagen since this substance stains green with the Masson trichrome method. Both of these stains show a marked similarity between colloid and the material described. It is admittedly an attractive

hypothesis that the material is colloid liberated from the damaged follicles of Hashimoto's disease. This, however, seems not to be the case since colloid stains intensely with P.A.S. and the material in question poorly or not at all. Furthermore, similar,if not identical material, has been seen in chronic inflammatory lymph draining regions remote from the thyroid and this further strengthens the hypothesis that the material is not colloid. The Feulgen reaction is entirely negative and shows clearly that the material is not nuclear in origin. Amyloid seems likely in view of the chronicity of the disease and the vigorous antigen-antibody reactions which occur so constantly. Unfortunately the material does not have the tinctorial properties of amyloid and it is not deposited on capillary walls or within the reticulin framework. The possibility remains that it could be fibrin especially since it stains red with Lendrum's acid picro-Mallory method. On the other hand the classical methods for demonstrating fibrin give equivocal results and one believes that the red colour obtained with Lendrum's method is simply an expression of the affinity of the material for acid dyes such as acid fuchsin.

Material with rather similar tinctorial properties is seen in the alveoli and bronchioles of some patients dying from cardiac failure. In this instance the material is clearly a plasma transudate and therefore it seems likely that the material seen in the germinal centres is a plasma protein. Since numerous plasma cells were seen in sections containing the red material it seems probable that it may in fact be a globulin.

The role of this distinctive material in the physiology of the lymphoid tissue is not understood but one might speculate that it is an antibody globulin secreted by the cells of the reticulo-endothelial system.

Summary and Conclusions.

1. Lymphadenoid goitre is a progressive disease which may terminate in severe fibrosis and myxoedema.

2. Basement membrane damage is a fundamental change and may occur in the absence of leucocytic infiltration. Leakage of colloid, through faulty basement membrane, results in a chronic inflammatory response and production of antibodies which aid in the phagocytosis of thyroglobulin.

3. A curious fuchsinophilic material is found frequently in the lymphoid follicles. The nature and function of this material is not understood.

CHAPTER I.
(Section II).

IMMUNOLOGY OF LYMPHADENOID GOITRE AND MYXOEDEMA.

These two conditions are discussed together since lymphadenoid goitre may lead to myxoedema and the histological changes in the gland are not infrequently qualitatively similar to both. The purpose of this section is to describe the frequency with which thyroid antibodies are found in these two conditions, to assess the relative value of different serological techniques in clinical diagnosis and discuss briefly the nature of the antigen involved.

Material:

Lymphadenoid goitre: All 20 patients were diagnosed following biopsy of the thyroid gland and were graded on histological appearances as previously described. In this small series there were no examples of fibroid goitre.

Primary Myxoedema: 19 of 21 patients were diagnosed as primary myxoedema in the Endocrine Clinic of Edinburgh Royal Infirmary and most of these were under treatment with thyroid when serum was taken for antibody tests. The other two patients were elderly females who died from myxoedema and

bronchopneumonia. In these two instances the thyroid gland and serum were obtained at necropsy. Both of these glands weighed less than 10 grams and there were no periglandular adhesions. Microscopic examination showed dense fibrosis, small islands of atrophic follicles, Askanazy cell change and small inconspicuous foci of lymphocytes and occasional plasma cells.

Secondary Myxoedema: Only four patients were available for study and again they were referred from the Royal Infirmary Endocrine Clinic.

Methods:

(Full technical details are given in the appendix).

The serum of each patient was examined for thyroglobulin antibodies by the tanned cell haemagglutination and precipitin techniques. The tanned cell haemagglutination method (Boyden, 1951) depends on the agglutination of erythrocytes coated with antigen when exposed to the appropriate anti-serum (Fig. 21).

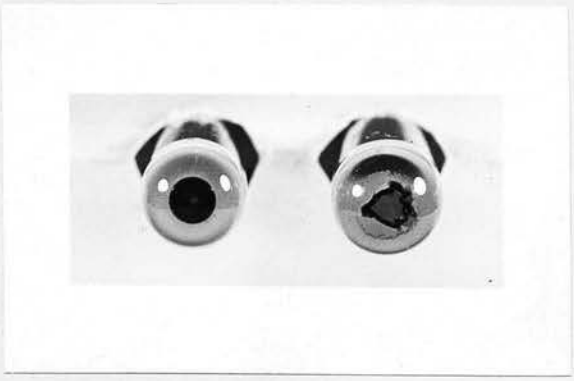


Fig. 21. Positive haemagglutination test: The tube on the left is a negative control and shows a compact button of cells. The tube on the right shows a carpet of agglutinated cells with a crenated well defined border.

The tanned cell inhibition test was sometimes used to make quite sure that the antibody in a particular serum was in fact an anti-thyroglobulin. The test serum is mixed with purified thyroglobulin (antigen). This removes the anti-thyroglobulin and when this serum is used in the ordinary haemagglutination test the titre is either zero or very much lower than that obtained with unabsorbed serum.

The precipitation methods used were layering of antigen of serum, a modified Oudin technique (fig. 22) and the Ouchterlony plate (fig. 23).

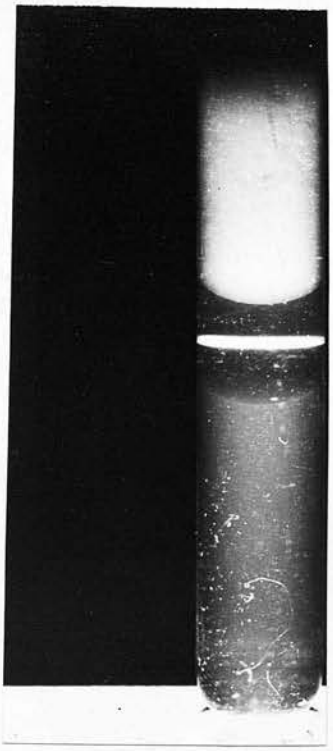


Fig. 22. Positive precipitin test: The antibodies diffuse upwards from the serum-agar column into the neutral zone. Antigen diffuses downwards from the antigen-agar column and forms a precipitate with antibody. The bands usually form in the neutral agar zone but with weak sera a broad diffuse band forms just above and within the serum-agar column.

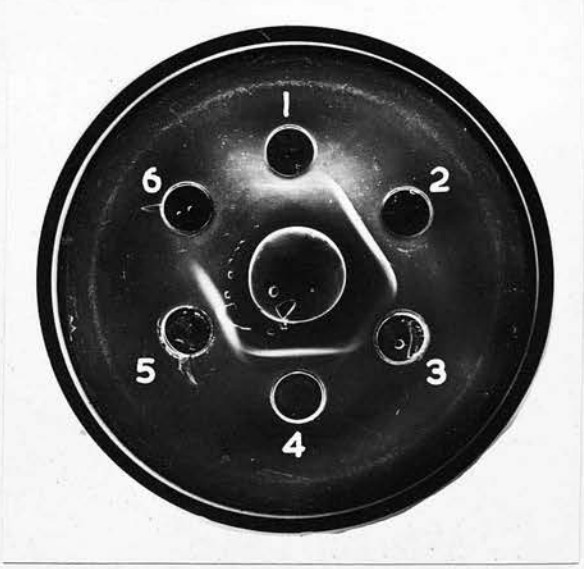


Fig. 23. Ouchterlony plate: Patients' sera are placed in the small wells labelled 1,2,3,4,5,6. The central well contains thyroid extract. Sera 2,3,4, 5 show typical positive reactions. Serum 1 shows a weak reaction and serum 6 is negative. Note the small spur at junction of precipitation bands between sera 1 and 2.

The term "precipitin test" in this paper means results obtained with the Oudin technique unless stated otherwise.

Results:

1. The frequency with which thyroid antibodies are found by the methods described are shown in tables II and III .

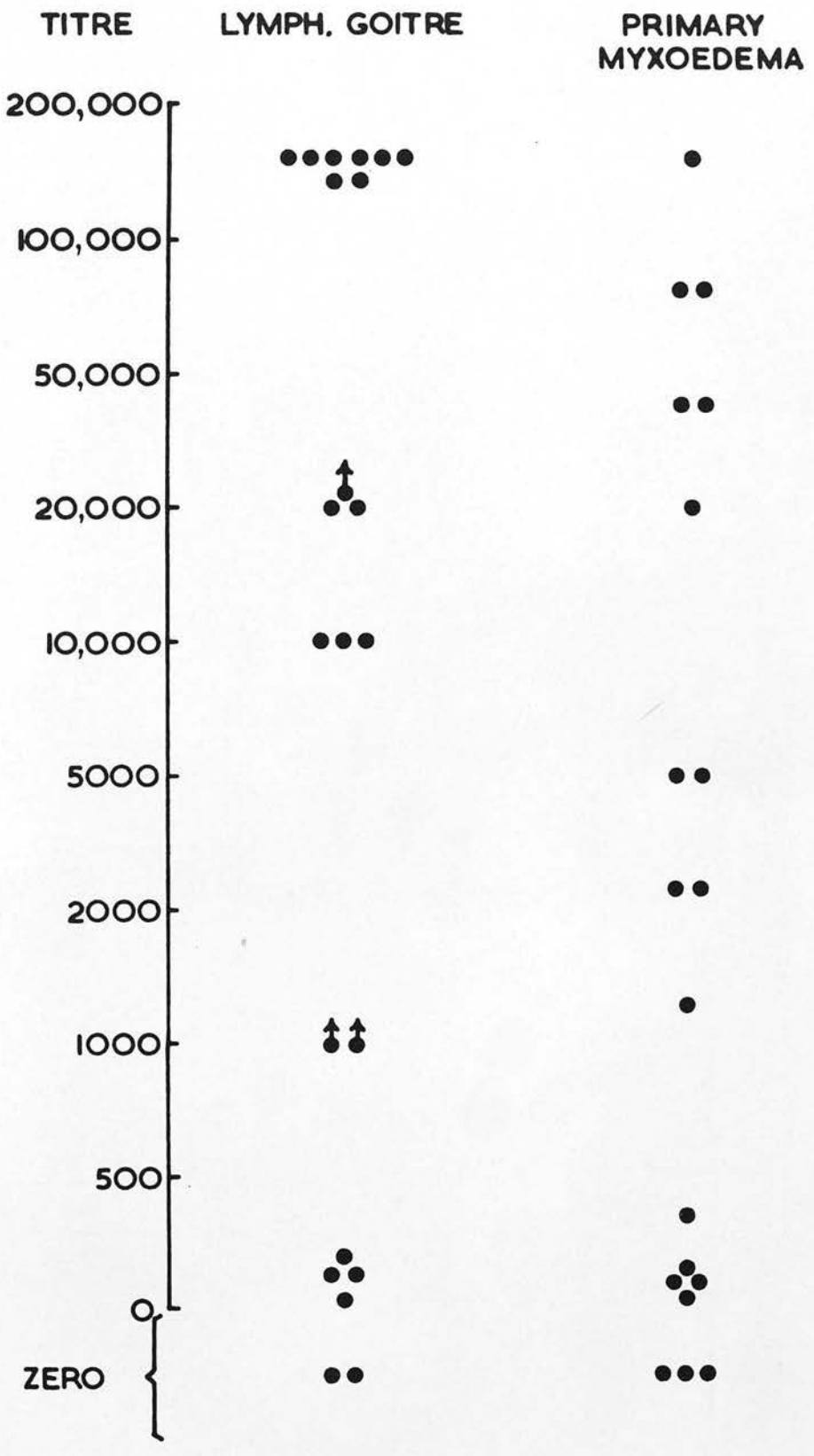
Table II .

	Lymphadenoid Goitre.		
Classification	No. of cases.	T.C.H.no. of positive	Precipitin no. of positive.
Lympho-epithelial	15	13	12
Lympho-fibroid	5	5	4
Fibroid	-	-	-

Table III .

	Primary and secondary Myxoedema.		
Classification	No. of cases.	T.C.H.no. of positive	Precipitin no. of positive.
Primary myxoedema without histology	19	16	4
Primary myxoedema with histology.	2	2	-
Secondary myxoedema	4	-	-

TANNED CELL HAEMAGGLUTINATION TEST



KEY: TITRE = Reciprocal of highest dilution of serum giving a positive reaction.

● = One patient

2. The height of the titres obtained by the tanned cell haemagglutination method is shown in fig. 24.

3. A few Hashimoto sera gave more than one precipitation band when tested against crude thyroid extract in an Ouchterlony plate. Fig. 25 shows two distinct bands.

Fig. 25.

Bands formed by one Hashimoto serum in the top two wells and antigen in the lower well.

Discussion:

Perhaps the most important feature is the presence of antibodies in the lympho-epithelial and lympho-fibrous phases of lymphadenoid goitre and also in the small fibroid glands of primary myxoedema which were examined at necropsy. One is surprised at the presence of antibodies in the fibroid phase since histological examination showed that very little thyroid tissue remained to furnish an antigenic stimulus. Had more cases of this kind been available it seems likely that only a proportion would show an immune response, the others being immunologically exhausted. There is also a striking similarity between the antibody patterns in lymphadenoid goitre and myxoedema, the main difference being the lower incidence in the latter.

Although the finding of antibodies in these two conditions lends support to the view that the small fibrous gland of myxoedema may be the late stage of Hashimoto's disease there are objections to accepting this without reservation. Firstly, the gland of myxoedema is usually small, whereas Hashimoto's disease presents as a goitre,

and in this series no glands of intermediate size have been observed.

This observation was not sustained by Williamson and Pearse (1925) who stated in a happy contradiction of terms that lymphadenoid goitre could be non goitrous. This has been the experience of others and Goudie et al (1957) found every gradation of size between the small gland of myxoedema and that of lymphadenoid goitre. It seems therefore that the different sizes of the gland in primary myxoedema and struma lymphomatosa does not preclude the same pathogenesis. Although the histological features of pink cell change, small follicles, lymphoid infiltration and fibrosis, may occur in the atrophic gland of myxoedema and struma lymphomatosa, one must remember that the response of thyroid gland to injury is limited, and that a common histological picture may be produced by different diseases.

The presence of thyroid antibodies is indicative **not** of any particular disease but of a biological reaction, namely auto-immunisation against thyroglobulin. This process may be started by a number of entirely different causes and it will be shown later that these antibodies

may be found in such diverse conditions as thyrotoxicosis and subacute thyroiditis. Although the evidence for identity of pathogenesis in some cases of myxoedema and struma lymphomatosa is strong, one may only conclude that a final decision is still to be reached.

The relative merits of precipitation and tanned cell methods.

The modified Oudin technique of precipitation in an agar gel has the great advantage in that positive reactions are clearly visible. When liquid antigen is layered onto serum an opalescent turbid zone sometimes develops at the interface when no antibodies are present. In such instances an erroneous positive result may be recorded. On the other hand this method gives results within minutes whereas the agar diffusion technique required 24 hours or several days before positive reactions become visible.

The Ouchterlony plate offers the benefit of testing several sera at once and therefore has a distinct advantage in economy of time and materials when large numbers of specimens are handled. Some extracts of thyroid gland have

shown an alarming tendency to spontaneous precipitation in this type of plate and for this reason the author prefers the Oudin tube method.

The precipitin technique gives far fewer positive results than the tanned cell method. This is not surprising when one remembers how very sensitive the tanned cell method is. A patient with a high tanned cell titre usually, but not always, gives a positive precipitin test. There are exceptions to this rule and three patients gave positive precipitin tests and very low titres with tanned cells. These low titres were obtained when purified thyroglobulin was used to coat the cells, and crude thyroid extract gave negative results. This finding would suggest that in some instances the precipitating antibody is different from the tanned cell reacting antibody.

The titres in lymphadenoid goitre were usually very high (fig. 24) and some sera gave a positive reaction in dilution of 1 in a million. High titres are characteristic of lymphadenoid goitre and with the exception of myxoedema occur but very rarely in any other type of thyroid disease.

The tanned cell technique suffers from

occasional lack of reproducibility of results but provided the test is done regularly and in exactly the same way each time, the results obtained are surprisingly constant. This technique, however, is too lengthy and elaborate to justify its use in a routine laboratory. Its sensitivity is such that antibodies can be detected in a wide variety of thyroid diseases and its specificity in lymphadenoid goitre rests solely on the very high titres obtained. Accordingly, its place in thyroid serology is that of a research tool and not a routine diagnostic aid.

The Nature of the Antigen.

It was first shown by Witebsky and Rose (1956) that thyroid antibodies had an unusually high degree of organ specificity and in the present work positive precipitin tests were obtained only with extracts of thyroid gland and not with other organs. Saline extracts of thyroid are unusual in that they contain a high proportion of one constituent, namely thyroglobulin, and therefore, it seems likely that this is the antigenic material in auto-immune thyroiditis. There appears to be more than one thyroglobulin and

Derrien, Michel and Roche demonstrated by salting out procedures the presence of several thyroglobulin fractions. The purified thyroglobulin used in this study was virtually homogeneous on electrophoresis although ultra-centrifugal analysis showed it was only 88% homogeneous and that two other small components were present. This chemical evidence for the existence of more than one thyroglobulin is supported by the immunological evidence of more than one antigen. The plurality of antigens is seen in fig.25 , where at least two and possibly three bands of precipitation develop when a particular Hashimoto serum was tested against crude thyroid extract in an Ouchterlony plate.

Roitt, Campbell and Doniach (1958) pointed out that as judged by the Ouchterlony test the antigens present in crude extract are also present in purified thyroglobulin. By immune electrophoresis in agar they showed the antigens in the crude extract had identical mobilities with that of thyroglobulin. They prepared a rabbit antiserum to purified human thyroglobulin and placed this antiserum with a Hashimoto serum known to give three lines in an Ouchterlony plate with thyroglobulin in the central well. The

precipitin curves merged completely which suggests that the same antigens were responsible for stimulating production of antibody in both rabbit and man. Accordingly, it seems likely that the antibody detected in man by precipitation and tanned cell haemagglutination tests,is,in fact,thyroglobulin.

The Value of Serologic Methods in clinical diagnosis.

The clinical problem is whether a lump in the neck is a simple goitre, a lymphadenoid goitre or malignancy. The precipitin test is never positive in simple colloid goitre, is frequently positive in struma lymphomatosa and may very occasionally be positive in malignant disease of the thyroid. Prior to the development of serologic methods the presence of a raised serum gamma globulin or abnormal liver function tests (Fromm et al, 1953; Luxton and Cooke, 1956) have been taken to support the diagnosis of lymphadenoid goitre. A comparison of liver function tests, serum gamma globulin levels and the precipitin test is shown in fig. 26 .

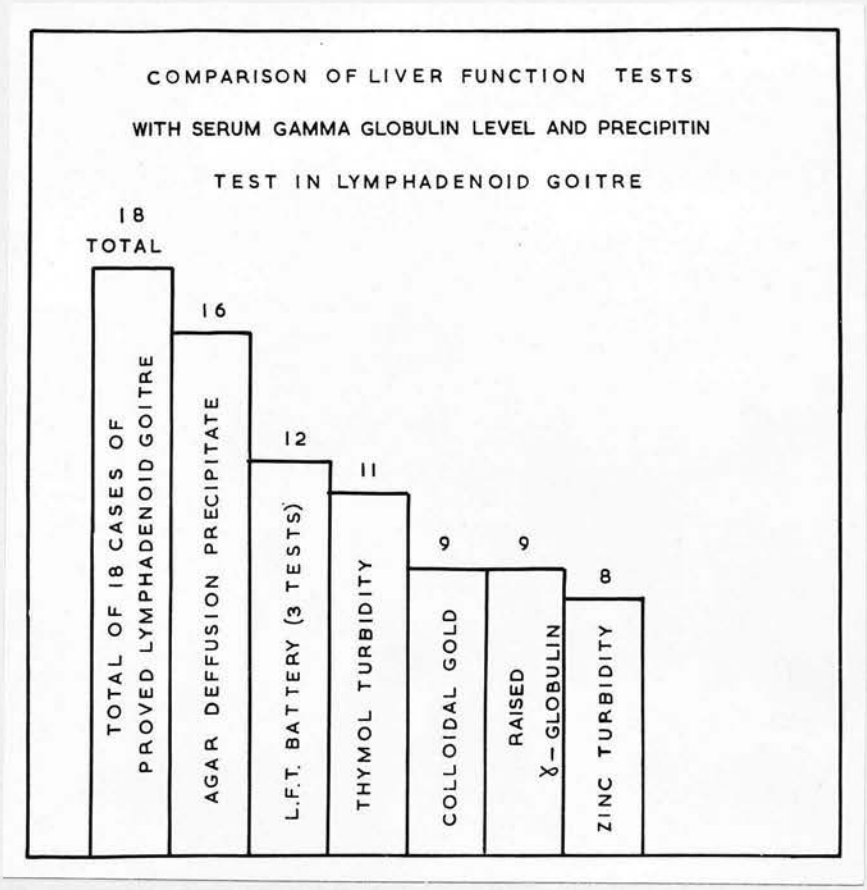


Fig. 26.

This series of patients is different from those recorded in fig. 24.

This indicates that the precipitin test is a more sensitive index of the presence of lymphadenoid goitre than any of the accepted biochemical tests. This finding of positive precipitin tests in Hashimoto's disease does not agree with the observations of Paine, Terplan, Rose, Witebsky and Egan (1957). These authors demonstrated antibodies in fourteen of seventeen cases of chronic non specific thyroiditis but failed to find them in fourteen cases of Hashimoto's disease. This discrepancy is probably not due to any basic difference in the transatlantic pathology of the disease but reflects rather the lack of unanimity between pathologists on the essential histological features of struma lymphomatosa. For example plasma cells were commonly seen in our material, but their presence would render the diagnosis of Hashimoto's disease unacceptable to these investigators.

On the other hand Doniach and Roitt (1957) found positive precipitin tests in twenty-five out of thirty patients with Hashimoto's disease; two of the five patients with negative results had been treated for a long time with thyroid extract and one other had undergone thyroidectomy.

Rather similar findings were reported by Goudie, Anderson, Gray, Clark, Murray and McNicol (1957) who noted positive precipitin tests in twenty of thirty cases of Hashimoto's disease although in only fifteen of these patients was the diagnosis confirmed by biopsy. These authors concluded that although the precipitin test was a simple and useful diagnostic method it was not sufficiently sensitive to supersede serum flocculation tests or the estimates of gamma globulin levels but should be used in conjunction with them. This has not been our experience which suggests the precipitin test is a more reliable diagnostic aid than any biochemical procedure.

Chapter II. (Section I.)

THE RELATION OF THYROID MALIGNANCY TO LYMPHADENOID GOITRE.

Despite the widespread belief that Struma lymphomatosa is entirely benign an increasing number of reports of co-existent neoplasm appear in the literature. Reticulum cell sarcomata arising in lymphadenoid goitre have been reported by Vaux (1936) and Cureton et al.(1957). A more cautious attitude was adopted by Kellet and Sutherland (1949) who recorded five cases of primary reticulum cell carcinoma of the thyroid in elderly women. They concluded that although the tumour arose in hyperplastic lymphoid foci it did not necessarily follow that the lymphoid tissue was part of a lymphadenoid goitre.

Lymphosarcoma has been described by Dinsmore (1949) and Walt et al (1957). The latter authors studied 18 cases of primary malignant lymphoma of the thyroid gland and found two cases where lymphadenoid goitre was also present.

Dailey et al (1955) found 37 carcinomas in 302 glands which showed the features of Struma lymphomatosa. Crile and Fisher (1956) noted the

simultaneous occurrence of thyroiditis and papillary carcinoma in two young women. In one of these cases the changes were "typical of lymphoid thyroiditis sometimes called lymphadenoid goitre". More recently (Lancet, 1958) Stuart, A.E. and Allan, W.S.A., described a case of indubitable thyroid malignancy where the uninvolved part of the gland showed changes indistinguishable from those seen in lymphadenoid goitre.

The relationship of thyroid neoplasia to lymphadenoid goitre assumes considerable importance in view of the recent tendency to treat all cases of Struma lymphomatosa conservatively. This viewpoint states that surgery now plays no part either in the recognition or cure of the condition and this change of clinical attitude has been endorsed by Hubble (Honeyman Gillespie Lecture, Edinburgh University, 1958).

The criteria used to establish a histological diagnosis of lymphadenoid goitre in a malignant thyroid are of the greatest importance. Firstly there should be extensive areas of undoubted lymphadenoid goitre in places remote from the

tumour. One has noted frequently a zone of chronic thyroiditis at the advancing edge of a neoplasm. This zone may contain lymphocytes, plasma cells, disrupted follicles and even areas of Askanazy cell change. When the biopsy material is limited in amount some have assumed that such changes are representative of the rest of the gland. This assumption naturally leads to false impression of the frequency with which lymphadenoid goitre and tumour co-exist. Secondly there should be clear evidence of undoubted malignancy in the affected part. There is a small group of thyroid lesions where the diagnosis of malignancy is attended with great difficulty. This difficulty has been experienced by the author in glands showing a diffuse infiltration of poorly differentiated tumour-like cells associated with a granulomatous inflammatory cell reaction. A survey of departmental records showed,in many cases, a curious disparity between histological diagnosis and the outcome of the disease. Some of these cases showed lymphadenoid-like changes but they are not included in the present study because of uncertainty over their precise histological nature. Accordingly, only two cases are reported here

both of which show the presence of tumour and lymphadenoid goitre in areas remote from the neoplasm. A third case which fails to meet the criteria previously mentioned, is also presented as an illustration of the inherent difficulty of the subject.

Case I.

Female aged 73 years. This patient consulted her doctor because of a swelling of one week's duration in the neck. The swelling was painless and there was no difficulty in swallowing or breathing. On examination a firm hard mass was present in the left lobe of the thyroid gland. There were no palpable cervical or supra-clavicular lymph nodes. At operation the goitre was densely adherent to the strap muscles and neighbouring tissues, and a course of radiotherapy commenced several days later. She died twelve years later at the age of 85 years. Her doctor certified the cause of death as senility and even at that time he could find no evidence of recurrence.

Fig. 27. (H.E. x ?).

Tumour, infiltrating muscle.

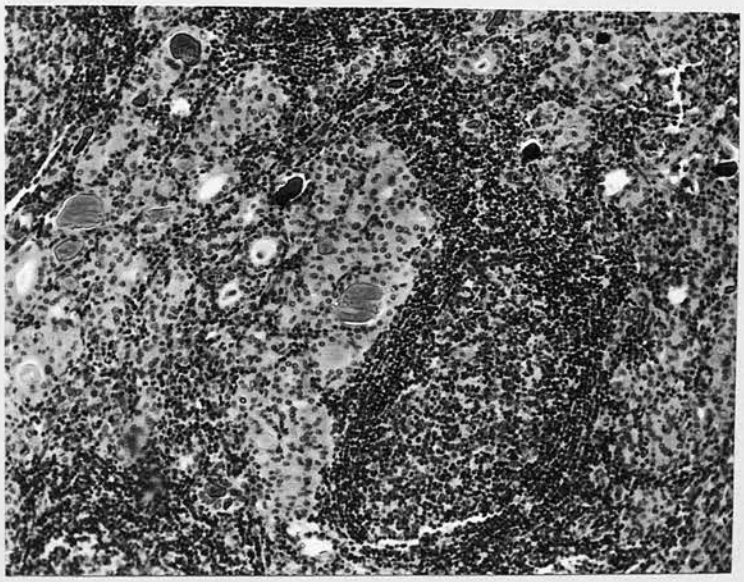


Fig. 28.

Area of lymphadenoid goitre showing small follicles, diffuse Askanazy cell change and lymphoid infiltration.

Case II.

(For which I am indebted to Professor J.S. Young, of Aberdeen University).

Female aged 34 with enlargement of 5 months duration of left lobe of thyroid. The lobe was firm and showed no fixation to superficial structures. No follow up is available.

The histological features are shown in Figs. 29 and 30.

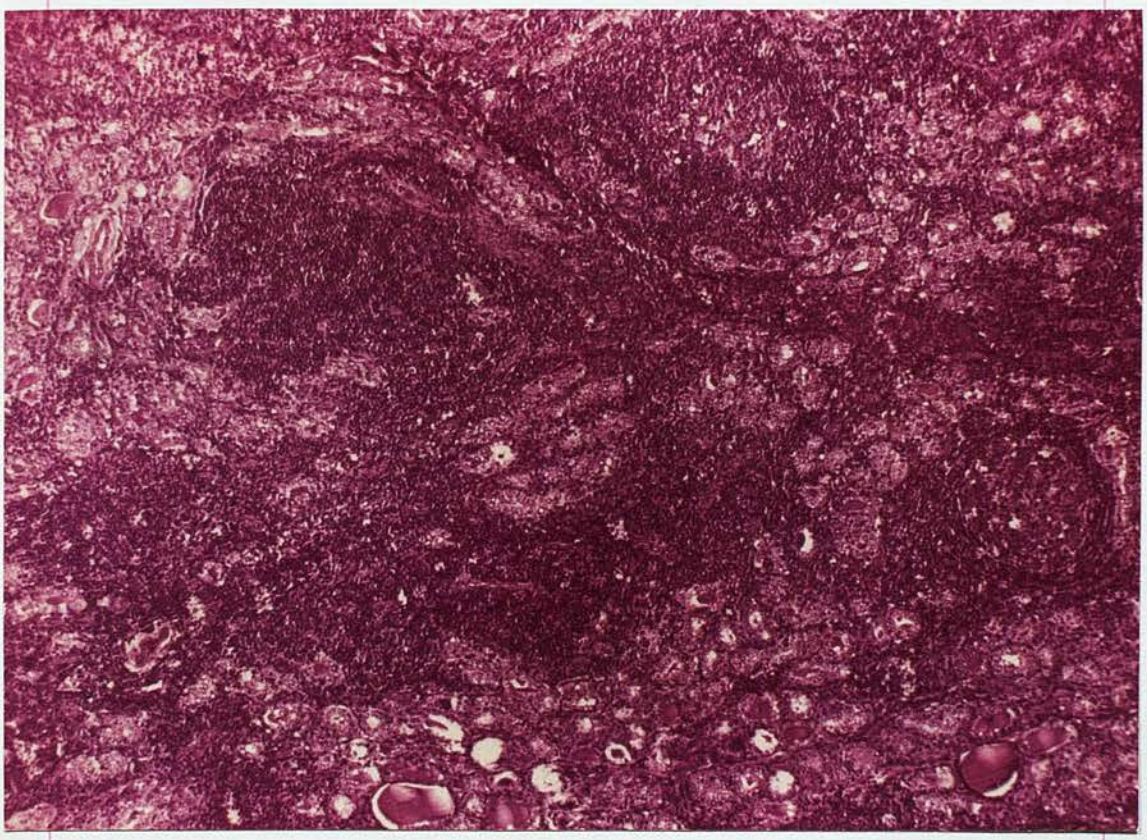


Fig. 29. (Case II).

Remarkable lymphoid hyperplasia affecting the non-neoplastic tissue, identical with Hashimoto's disease.

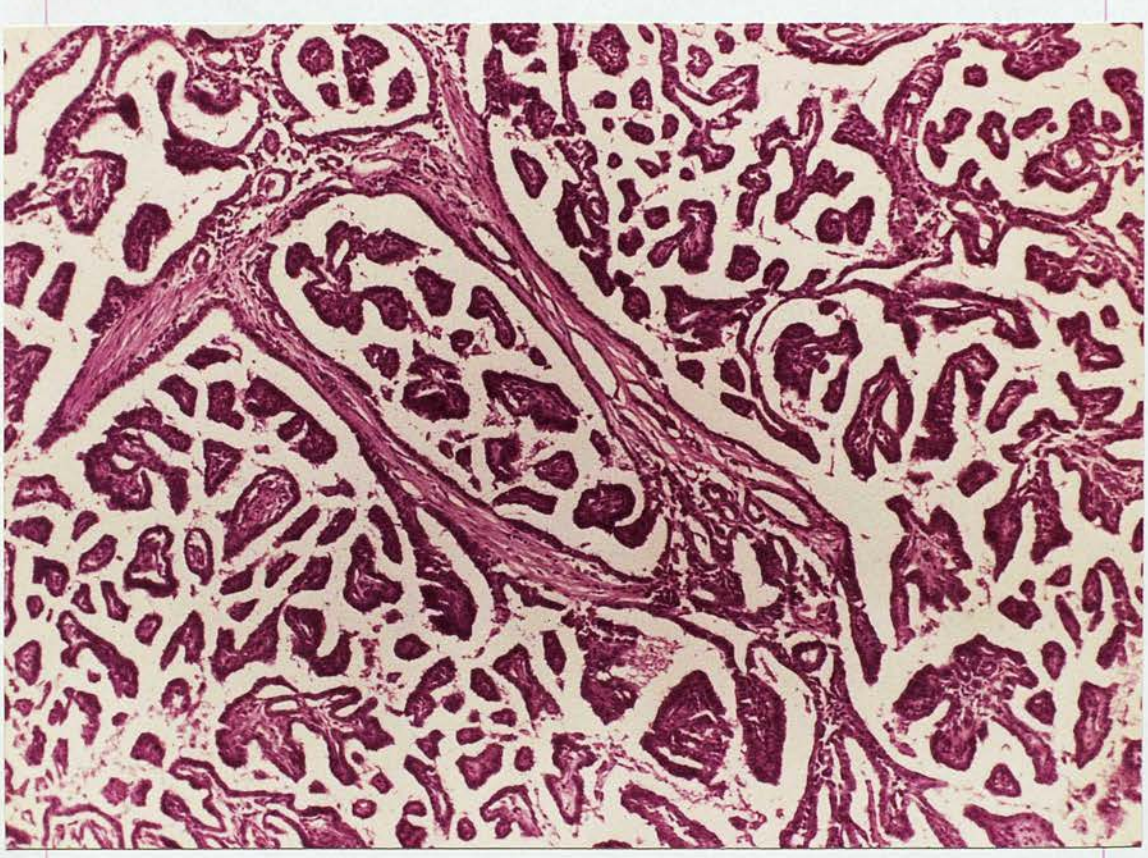


Fig. 30. (Case II).

Papillary adenocarcinoma.

Case III.

Female aged 65 years. She had been aware of a small hard lump on the right side of the neck for many years. This lump had recently increased in size and clinical examinations showed a diffuse hard swelling affecting the right lobe and isthmus. The gland was resected and radiotherapy started. She died a few weeks later from widely disseminated malignant disease.

The histology of the thyroid gland is shown in Figs. 31 and 32.

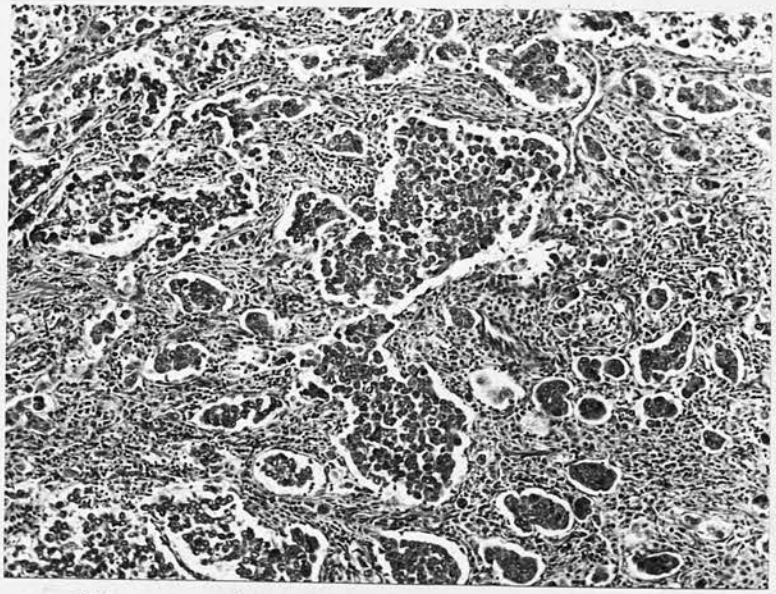


Fig. 31 . (Case III)

Anaplastic carcinoma.

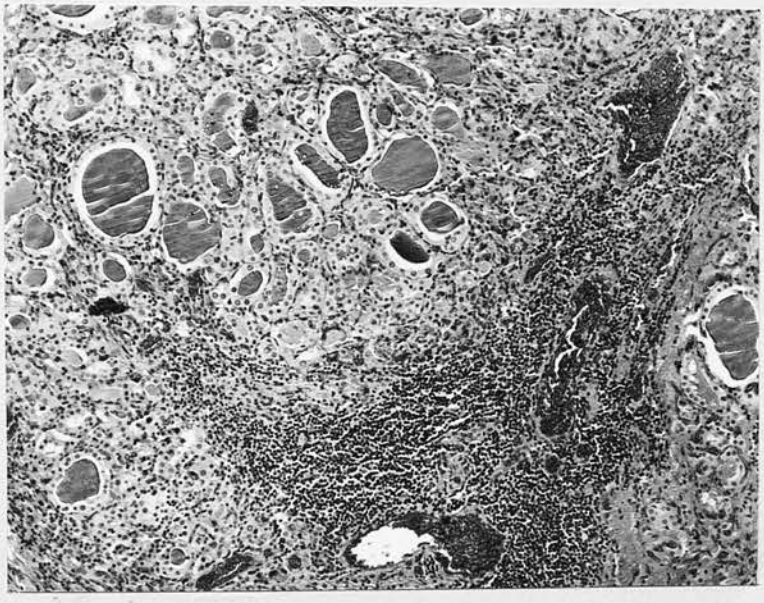


Fig. 32 . (Case III).

Extensive diffuse Askanazy change with scanty lymphoid infiltration. This is not regarded as lymphadenoid goitre owing to minor character of lymphoid infiltration, absence of basement membrane change, and proximity to advancing edge of tumour. This assessment may be too conservative but if a loose interpretation of the histology of struma lymphomatosa is permitted then the original concept entirely loses its meaning.

Discussion.

It is significant that none of the 100 cases of lymphadenoid goitre described earlier in this thesis showed unsuspected co-existent malignant disease. On the other hand cases showing such changes were usually,but not always,diagnosed clinically as malignant,and the changes of Hashimoto's disease were incidental findings in the histopathology.

It is not easy to determine whether the lymphadenoid change is the cause or result of malignancy. When only a small piece of uninvolved tissue is available for examination one hesitates to ascribe the degenerative and inflammatory changes to a previously existing lymphadenoid goitre. On the contrary it seems possible that these changes are caused by lymphatic obstructions and direct mechanical pressure by tumour on normal acini. Accordingly, only cases showing diffuse Struma lymphomatosa in relatively large areas remote from the tumour should be accepted. Considerable difficulty is experienced when one encounters microscopic foci of Hashimoto's disease embedded in a mass

of neoplasm. This has been our experience on several occasions but in no instance were these changes said to be representative of the thyroid before the onset of neoplasia. Crile and Fisher (1956) believe that in one of their two cases papillary carcinoma supersedes lymphadenoid goitre and Struma lymphomatosa would seem to have been co-existent with tumour in the two cases reported here, although it is clear the problem is insoluble on histological grounds alone. The requirements for the establishment of lymphadenoid goitre as a precursor of malignancy should include the presence of characteristic lump in the neck for many months or years together with the usual biochemical serological and metabolic abnormalities associated with the disease. This benign phase, confirmed by biopsy should then be followed by obvious malignancy established by microscopic examination of the gland.

The natural history of Struma lymphomatosa has not yet been written but the increasing use of conservative therapy will add to knowledge of complications of this disease. The frequency with which lymphadenoid goitre and malignancy co-exist is certainly low, and these

cases are presented as a warning that it may be unwise at the present time to discard the benefit of open biopsy.

A subject of more practical interest is the frequency with which carcinoma of the thyroid gives positive serologic tests which may lead to an erroneous diagnosis of lymphadenoid goitre. This problem is discussed later in the section on "Immunology of Thyroid Malignancy."

Chapter II. (Section II.)

THE RELATION OF LYMPHADENOID GOITRE TO MALIGNANCY OUTWITH THE THYROID GLAND.

Case I.

A female aged 59 years suffered from a goitre for many years. The gland was firm, mobile and diffusely enlarged. A subtotal thyroidectomy was undertaken and the lateral lobes on section showed a pinkish brown homogeneous appearance with a considerable increase in the fibrous stroma. The microscopic features were those of struma lymphomatosa (fig. 33a,b) and no evidence of malignancy could be discovered in the thyroid.

She made a good recovery and remained well for a year when she complained of weakness, shortness of breath and dragging pains in the left side of the abdomen. Clinical examination showed an ill patient with enlargement of liver and spleen, but no lymphadenopathy. Haematological investigations were as follows - Hb. 64%, C.I. 0.95, platelets 110,000, W.B.C. 14,000. Diff. w.b.c.: polymorphs 13%, lymphocytes 87%. No marrow obtained after two sternal punctures. Radiotherapy was given but she died within a few days. Death was preceded by severe vomiting and abdominal pain. The principal necropsy

findings were as follows.

The spleen weighed 1400 gm. and on section the lymphoid follicles stood out very clearly. The liver (1900 gm.) was firm, pale and showed obvious infiltration around the portal tracts. The small bowel was matted by fibrinous adhesions and on separating these adhesions small beads of pus exuded from the serous surface. Scattered over the whole of the small intestine but especially marked in the jejunum were small yellowish nodules under the serous surface. The mucosa was thickened and Peyer's patches showed hyperplasia. The mesenteric lymph glands were enlarged, had a rubbery consistency and on section were yellowish grey. The sternum, vertebral column and mid-shaft of femur showed greyish pink marrow with small areas of haemorrhage.

The remainder of the thyroid gland was firm and yellowish brown.

The microscopic findings showed marked peri-portal round cell infiltration in the liver (fig.34) The mesenteric glands were packed with small lymphocytes and larger mononuclear cells; the architecture was well preserved. The thyroid showed the features of lymphadenoid goitre and the

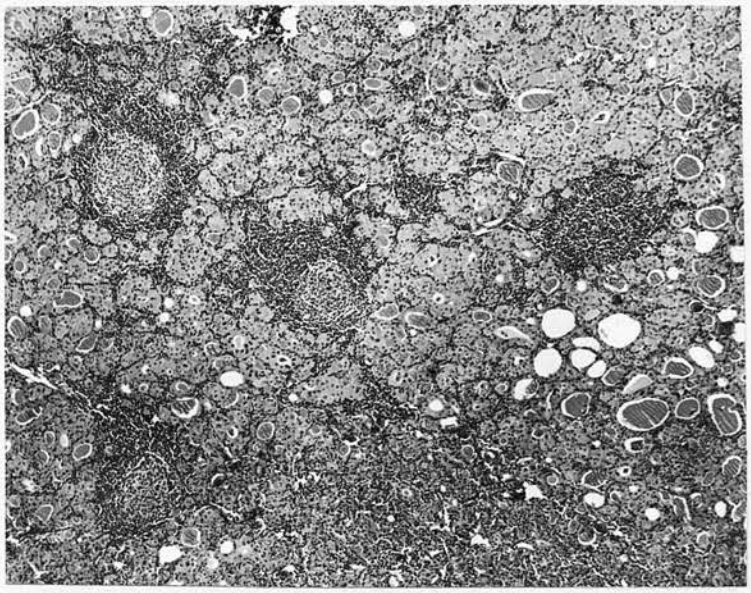


Fig. 33 (a). (H.E. x 45). (Case I.)

Struma Lymphomatosa.

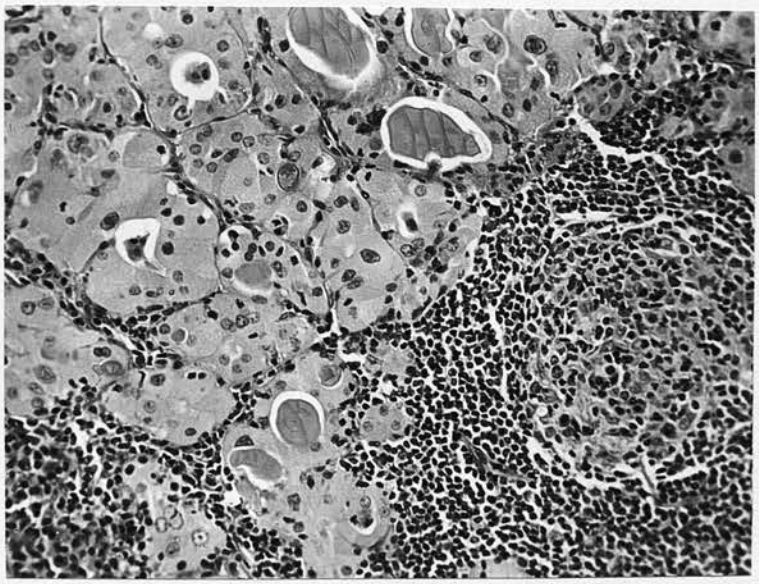


Fig. 33 (b). (H.E. x 210). (Case I.)

Same section.

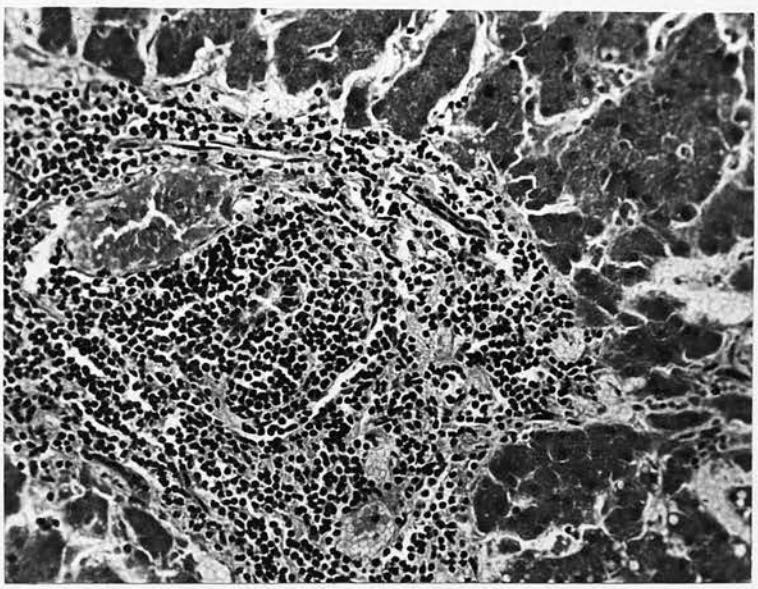


Fig. 34. (H.E. x 210). (Case I).

Liver, showing dense lymphocytic infiltration of portal tract.

bone marrow examined contained only fat.

Case II.

A male aged 41 years, complained of a swelling of six months duration in the neck. Clinical examination showed a diffuse thyroid enlargement. Chest films revealed the mass extended down to the level of the hila. Moderately enlarged lymph nodes were present in the left supra-clavicular region and right axilla. A test dose of radiotherapy produced marked shrinkage of the gland following which an open biopsy was done. On receipt of the pathologist's report that the thyroid showed the features of struma lymphomatosa (fig. 35) and that no malignancy was present,a further course of radiotherapy was given. Two months later the mediastinal mass recurred and he was very breathless. No thyroid enlargement could be detected and no glands were palpable. Further radiotherapy caused some improvement. A few weeks later he developed a large mass in the tonsillar region (fig. 36) and was again given radiotherapy following which he returned home, where subsequently a big lump rose in the right thigh. He died within a few days, and no necropsy was done.

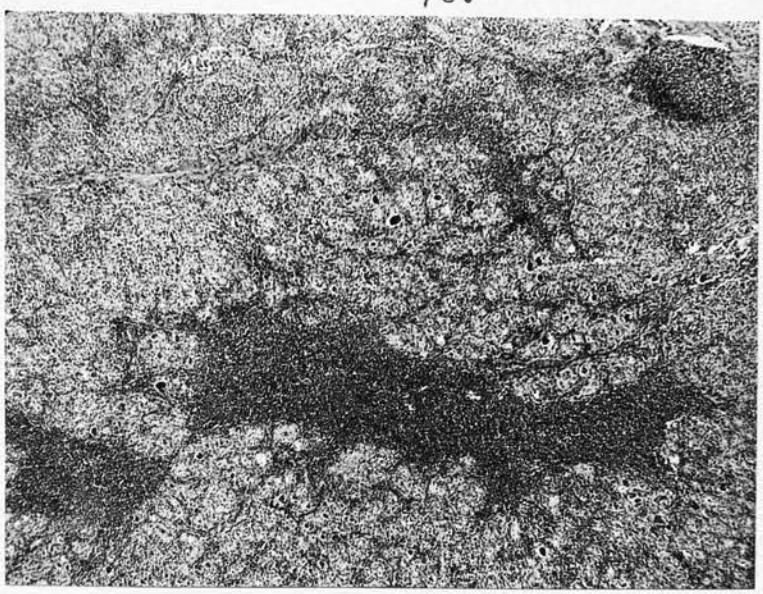


Fig. 35. (Case II.)

Thyroid biopsy showing small follicles and dense lymphoid infiltrations. The epithelium is cuboidal and does not show Askanazy cell change.

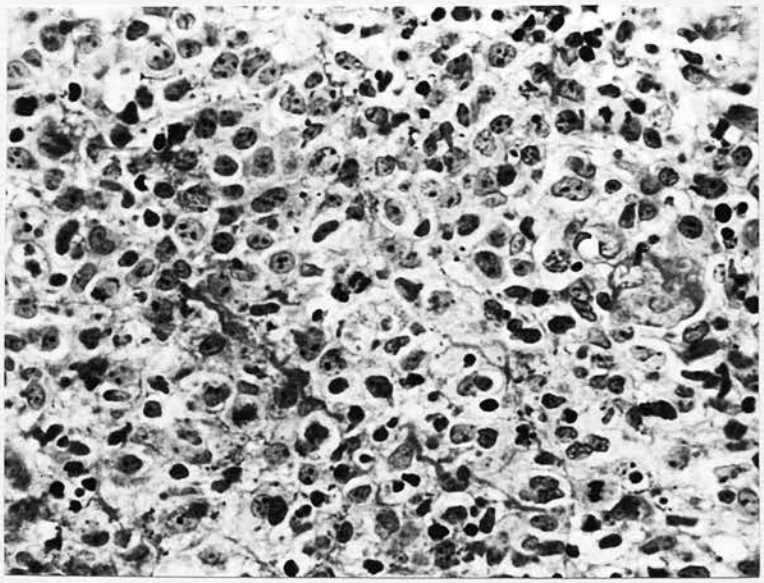


Fig. 36. (H.E. x 450). (Case II.)

Tonsillar mass showing reticulum cell sarcoma.

Case III.

A female aged 54 years complained of a painless swelling in the neck of two weeks duration. She had previously been in good health. Clinical examination revealed a diffusely enlarged firm and mobile thyroid. No glands were palpable and thyroid function was clinically normal. A diagnosis of struma lymphomatosa was made and confirmed by needle biopsy. Electrophoresis of her plasma protein showed a gamma globulin level of 1 gm. per 100 ml. (17% of the total protein). The colloidal gold reaction was zero and thyroid turbidity two units.

She was not given treatment, but was observed at close intervals. One year later it was noted that no change had taken place, in either her local or general condition. At this time a precipitin test using crude thyroid extract was done. This was the first occasion on which the author performed this test and a rapid flocculation occurred when the liquid antigen was gently layered onto the patient's serum. The tanned cell haemagglutination test gave a titre of over 1/1000 but was not measured to the end point. The serum was kept for use as a positive control in thyroid

antibody tests. Some two months later she underwent tonsillectomy but thereafter complained of weight loss, anorexia and lack of well being. She developed a swelling on the shoulder region and was readmitted to hospital.

Shortly before her admission a further sample of blood was taken for thyroid precipitin tests. Surprisingly, these were negative, and enquiry as to whether the blood sample had been incorrectly labelled, or taken from the wrong person, showed that no error had been made.

Clinical examination at this time showed the thyroid was quite unchanged and presented the same appearance as when first seen fifteen months previously. There was a firm, fixed gland in the right axilla, a hard mass over the left scapula and similar swellings in the right supra-clavicular region and over the right posterior superior iliac spine. An open biopsy of the thyroid gland showed the gross appearances of struma lymphomatosa, which was confirmed by the microscopic features seen in fig. 37 . Biopsy of the supra-clavicular mass showed a poorly differentiated tumour, illustrated in fig. 38.

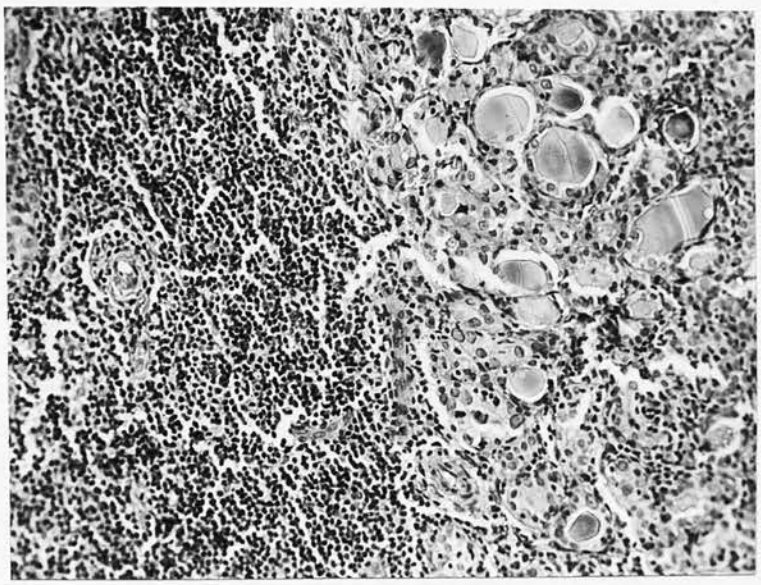


Fig. 37. (Case III).

Thyroid biopsy showing lymphoid infiltration, small follicles and Askanazy cell change.

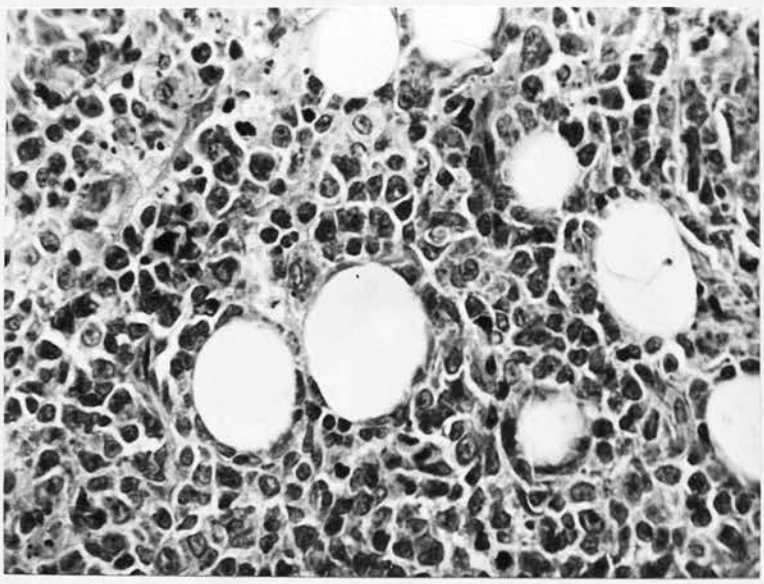


Fig. 38. (H.E. x 450). (Case III).

Biopsy of supraclavicular mass.

Discussion:

These three cases all showed the changes of struma lymphomatosa without discoverable malignancy in the thyroid gland. All succumbed within a short time to a malignant process whose clinical and pathological features were strongly suggestive of a reticulosis. Admittedly, the histological distinction between anaplastic thyroid carcinoma and reticulum cell sarcoma, or lymphosarcoma and small round cell carcinoma of the thyroid gland may be very difficult. Although the author believes these cases are examples of a neoplastic reticulosis, the important finding is the association of a malignant process outside the thyroid with changes in the thyroid of lymphadenoid goitre.

Case I showed the striking feature of peritonitis due to perforation of an infiltrated small bowel. This feature resembles the findings of Brewer and Orr (1953), who reviewed cases of undifferentiated thyroid tumours and found, in some, an unusually high incidence of involvement of the wall of the intestinal tract. They coined the term "Struma Reticulosa" and were not quite prepared to say whether their cases were

neoplastic or unusual variants of lymphadenoid goitre.

Case III is unique in that the precipitin test was positive at first but became negative when the neoplastic process advanced. Whilst this finding may be due to a chance fluctuation of antibody level resulting in lack of reproducibility of results, it seems more likely that destruction of the antibody forming mechanism by reticulum cell sarcoma is the answer.

It is uncertain whether lymphadenoid goitre predisposes significantly towards malignancy of this type. A useful field for future research would be the routine examination of all thyroid glands in patients dead from reticulosis,keeping in mind the proviso that not all glands showing the features of struma lymphomatosa are in fact goitres. The author believes the incidence of this type of malignancy in struma lymphomatosa is low, but only studies along the lines suggested can reveal the true frequency.

Chapter II. (Section III).

THE IMMUNOLOGY OF THYROID MALIGNANCY.

Thyroid antibodies associated with thyroid malignancy were first noted by Stuart and Allan (Lancet, 1958) and this observation was confirmed by Roitt and Doniach (Lancet, 1958) in a larger series of cases. The present study describes the presence of auto-antibodies in four of 9 cases with thyroid neoplasia.

Case I. (I.D.).

A housewife aged 65 years complained of difficulty in swallowing for several months. Examination showed a firm swelling of the thyroid which involved both lobes and the isthmus. Numerous enlarged lymph nodes were palpable on both sides of the neck. Otherwise physical examination was negative. By electrophoresis her serum gamma globulin showed a concentration of 1.3 grams per 100 ml., 24 per cent of her total serum protein. She gave a positive precipitin test to thyroid extract and the tanned cell haemagglutination titre was 20,000. The clinical diagnosis was carcinoma of the thyroid and an attempt was made to relieve the tracheal

obstruction surgically. It became apparent that relief was impossible and the operation was abandoned. The patient died 24 hours later and an autopsy was not obtained.

Pathological examination: The operation specimen consisted of three firm nodules of tissue, the largest measuring 3.2 x 2.1 cm. and the smallest 1 cm. in diameter. Microscopically the lesion was an anaplastic pleomorphic carcinoma with associated lymphadenoid change.

Micro: Anaplastic pleomorphic carcinoma (fig. 39).

Uninvolved areas: Many of the follicles were smaller than usual and the epithelium was pink, granular and of the Askanazy type. There were also a smaller number of follicles with a normal appearance. A moderate patchy infiltration by plasma cells and lymphocytes was seen but no lymphoid follicles were present. Marked fragmentation (fig. 40) of the acinar basement membrane was noted. Although selected microscopic fields showed changes indistinguishable from struma lymphomatosa, many of these areas were situated only a few millimetres from tumour. Accordingly it is not claimed that the neoplasm

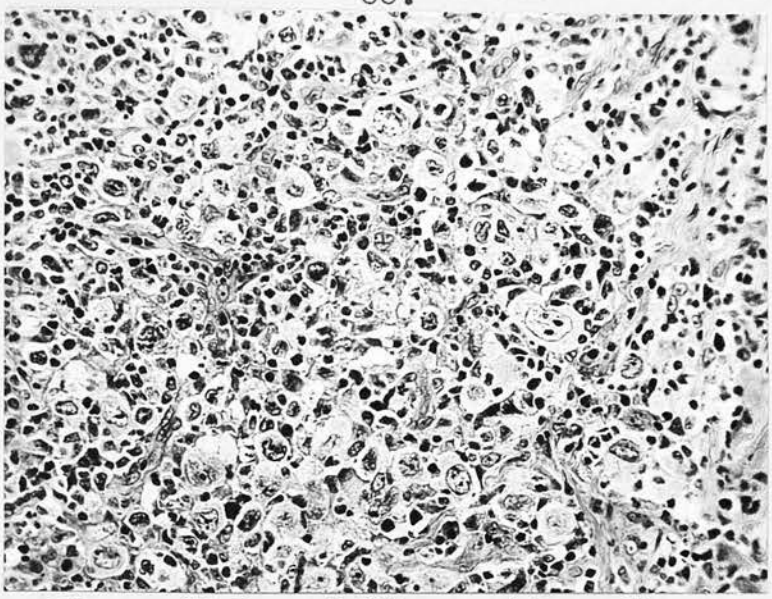


Fig. 39. (H.E. x 250). (Case I).

Anaplastic pleomorphic thyroid carcinoma.

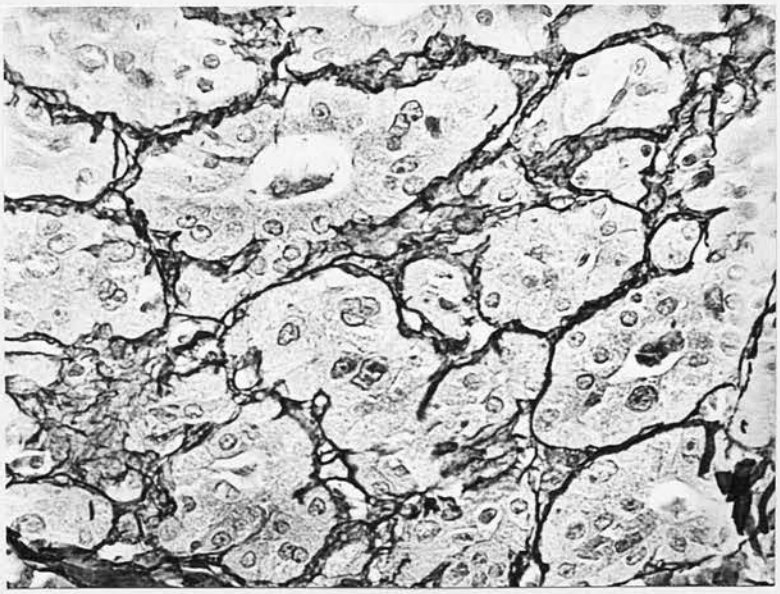


Fig. 40. (Silver x 380) (Case I).

Area not involved by tumour; marked basement membrane damage.

arose in a lymphadenoid goitre although admittedly the surviving parenchyma shows 'lymphadenoid-like' change.

Case II. (A.M.)

A housewife aged 60 consulted her doctor in 1954 because of inability to sing properly, when a diagnosis of a small "cyst" in the left lobe of the thyroid was made. In 1957 her attention was drawn to a further swelling this time in the right lobe of the gland. She was admitted to hospital a year later when a diffuse asymmetrical thyroid enlargement was noted, the right lobe being much larger than the left. The surface was smooth and the consistence hard throughout. There were no palpable cervical lymph glands. The remainder of the physical examination was negative and clinically thyroid function appeared normal. The clinical diagnosis was uncertain and rested between chronic thyroiditis and carcinoma.

Her serum cholesterol was 241 mgs. per 100 ml., and her serum protein bound iodine was 6.0 micrograms per cent; the 48 hour I_{131} uptake was 23 per cent of the dose and her 48 hour I_{131} excretion

was 66 per cent of the dose. The 48 hour protein bound plasma activity level was 0.2 per cent of the dose per litre of plasma. Serum colloidal gold reaction was negative, thymol turbidity 4 units and zinc turbidity 5 units. Serum electrophoresis showed a gamma globulin concentration of 1.0 grams per 100 mls., equivalent to 18 per cent of the total serum protein. The precipitin test with crude thyroid extract was positive and the tanned cell haemagglutination titre was 20,000.

The serological results suggested the possibility of lymphadenoid goitre and a biopsy was taken. The hardness of the gland was confirmed but the thyroid was not adherent to the strap muscles and did not appear malignant. Following the pathological report of malignancy a further operation was undertaken. On this occasion the left lobe of the gland, although somewhat enlarged, was soft and normal in appearance. The right lobe, however, was obviously malignant, densely hard, extending high up in the neck and low down behind the clavicle. It was firmly adherent to trachea and oesophagus and complete extirpation of tumour was impossible.

Pathological examination: The tissues examined consisted of two small pieces of thyroid removed at the initial biopsy and the excised lobe, a nodular ovoid mass, measuring 10 x 5 x 5 cm. and weighing 125 gms. Many large sections from different parts of these tissues were examined. Practically all normal tissue (fig. 41) was replaced by well differentiated papillary adenocarcinoma (fig. 42), which had invaded the extra-capsular tissues and metastasised to an adjacent lymph node. There were no areas of lymphadenoid change although there was a focus of lymphocytic infiltration in the only normal tissue seen.

Case III.(Mrs. M.)

This patient aged 57 had a solitary nodule diagnosed as an adenoma in 1950. During the past year this nodule increased steadily in size, and she complained of dysphagia. The clinical diagnosis was thyroid carcinoma and a malignant infiltration of the trachea was present. She died shortly after operation. Necropsy showed respiratory obstruction, bronchopneumonia and papillary thyroid carcinoma in cervical lymph

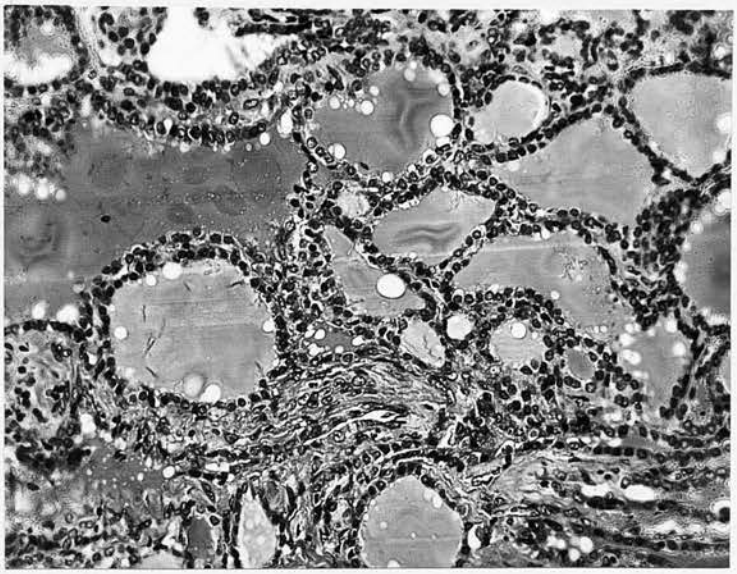


Fig. 41 . (H.E. x 200). (Case II).

Normal thyroid tissue.

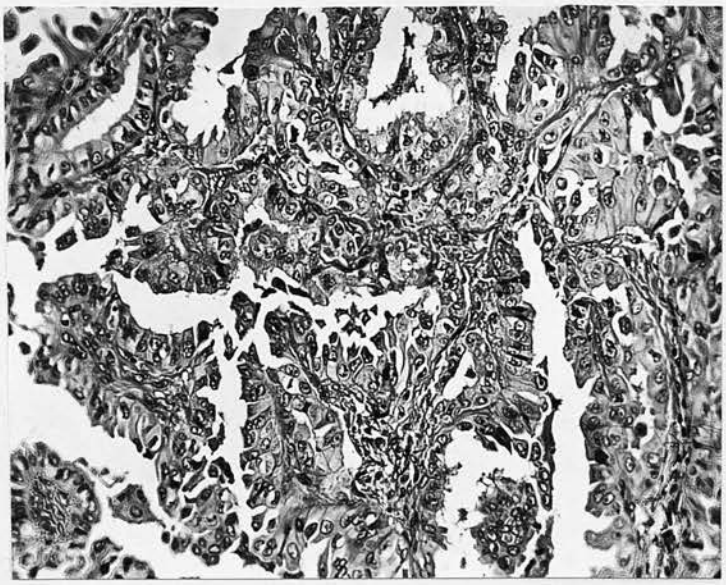


Fig. 42 . (Case II).

Papillary carcinoma.

nodes.

The precipitin test with crude thyroid extract was negative and the tanned cell haemagglutination titre was 640.

Pathological examination: The specimen consisted of a firm irregular mass of tissue weighing 92 gms. Much of the tissue was uniformly firm and white.

Micro: Anaplastic pleomorphic carcinoma (fig. 43).

Uninvolved areas: Many areas showed small follicles lined by cuboidal cells showing Askanazy change. A diffuse interstitial infiltration by lymphocytes and plasma cells was present and numerous lymphoid follicles were seen (fig. 44). Elsewhere there were small groups of entirely normal follicles where the epithelium in no way resembled that of Hashimoto's disease.

Case IV. (Mrs. M.W.)

Mrs. M.W. aged 72 years, had a malignant melanoma of toe removed six months before her admission to hospital with dysphagia. The clinical diagnosis was carcinoma of thyroid. The precipitin test was negative and the tanned cell

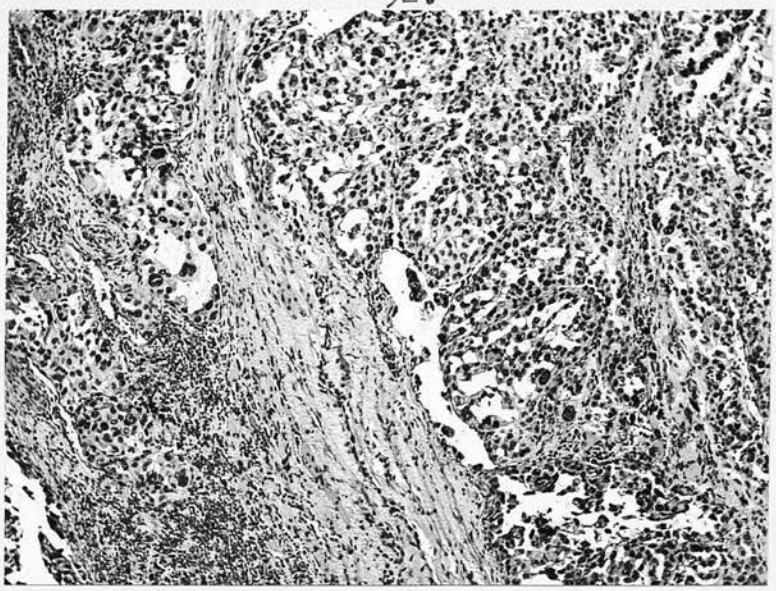


Fig. 43 . (H.E. x 75) (Case III).
Thyroid carcinoma.

Fig. 44 . (Case III).
Lymphadenoid-like area in thyroid gland.

test gave a titre of 640.

Pathological examination of the biopsy showed no thyroid tissue, only a highly cellular tumour with the appearances of metastatic melanoma, (fig. 45).

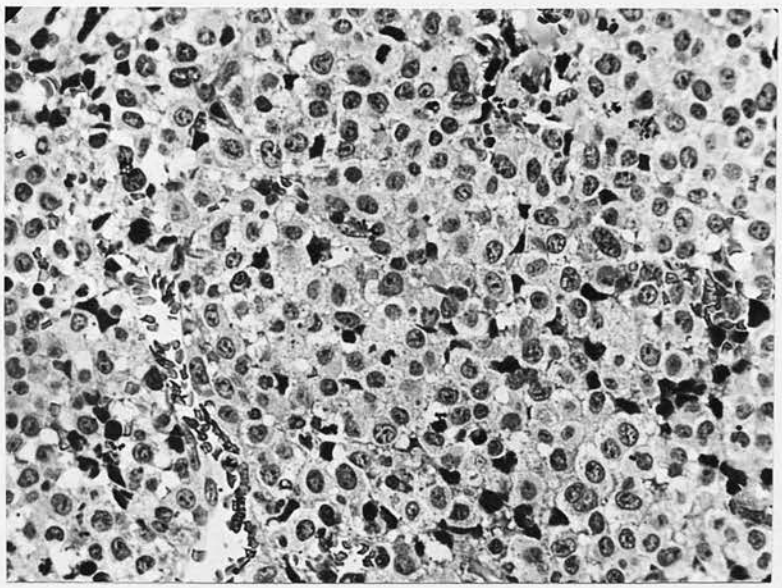


Fig. 45. (Case IV).
Malignant melanoma in thyroid gland.

Discussion:

All four patients had unequivocal microscopic evidence of carcinoma of the thyroid gland.

The non malignant areas of the gland showed 'lymphadenoid' changes in cases I and III, a relatively normal structure in case II and no thyroid tissue was found in case IV. Whilst co-existent lymphadenoid goitre may possibly have been responsible for thyroid antibodies in cases I and III, this is probably not the explanation in case II and seems unlikely in case IV, where the lesion was a metastatic melanoma. An alternative explanation of the finding of auto-antibodies in thyroid neoplasia is the release of antigenic thyroglobulin from follicles disrupted by invasive tumour. This would adequately account for the presence of antibodies in both primary and secondary tumours of the gland.

It is noteworthy that the precipitin test was negative on two occasions when the tanned cell method gave positive results. Roitt and Doniach (Lancet, 1958) found thyroid antibodies in 10 of 36 cases of thyroid malignancy when they used the sensitive tanned cell technique and none with the precipitin method. Since then Roitt mentions

in a personal communication the finding of a positive precipitin test in a patient with frank thyroid carcinoma.

The finding of auto-antibodies in thyroid neoplasia is disturbing in view of the increasing reliance placed on immunological reactions in thyroid disease. It might be thought that the clinical picture of thyroid carcinoma is so distinctive that the presence of positive precipitin tests would not lead to confusion with lymphadenoid goitre. While this may be so in specialised thyroid clinics, these two conditions are not common, and the clinical distinction between thyroid carcinoma and lymphadenoid goitre may offer considerable difficulty.

Until recently the ultimate diagnosis often depended upon microscopic examination of the gland. Positive precipitin tests may however, now be taken to favour a diagnosis of lymphadenoid goitre, and the finding of positive serological tests in thyroid carcinoma calls for caution in the interpretation of such evidence. In three of the cases reported here, the diagnosis of carcinoma was apparent clinically and no abnormal biochemical or serological findings could modify the diagnosis.

Roitt and Doniach (Lancet, 1958) make the point that when antibodies are found in cancer cases the clinical features are those of tumour and not of lymphadenoid goitre. Our own experience, in general, supports this view, but important exceptions are met with. For example in case II the true diagnosis was not apparent clinically and clinical management might have been modified by the discovery of serological changes suggestive of lymphadenoid goitre. It is, therefore, important that the finding of positive serologic tests should not necessarily lead to a firm diagnosis of lymphadenoid goitre. Lymphadenoid goitre may predispose to the development of malignancy and the two conditions may be present together. It seems virtually certain that more cases of this type will be reported in the future, and accordingly, surgical assistance should be freely invoked to establish a certain diagnosis.

Summary and Conclusions.

1. The co-existence of lymphadenoid goitre, with both carcinoma of the thyroid and extra thyroid malignancy, is described. The incidence is low but is probably higher than the few reports in the literature would suggest.

2. Although serological findings are strong confirmation of the presence of a lymphadenoid goitre, undue reliance should not be placed on them in the absence of open biopsy.

Chapter III. (Section I).

THE IMMUNOLOGY OF THYROTOXICOSIS AND SIMPLE NODULAR GOITRE.

SECTION I.

Material: 74 cases of thyrotoxicosis.

34 cases of non toxic nodular goitre

148 controls from hospital patients without thyroid disease.

50 controls from healthy blood donors.

Method:

Serum from each of these patients was examined by the tanned cell and precipitin methods.

Results:

1. Controls: Fig. 46 shows the results of the tanned cell haemagglutination method in the control series. The precipitin test was entirely negative in this group.

2. Thyrotoxicosis and Nodular Goitre: The results of the tanned cell test (T.C.H.) are shown in Table IV and the precipitin test in Table V . The titres obtained with the tanned cell method are illustrated in fig. 47 .

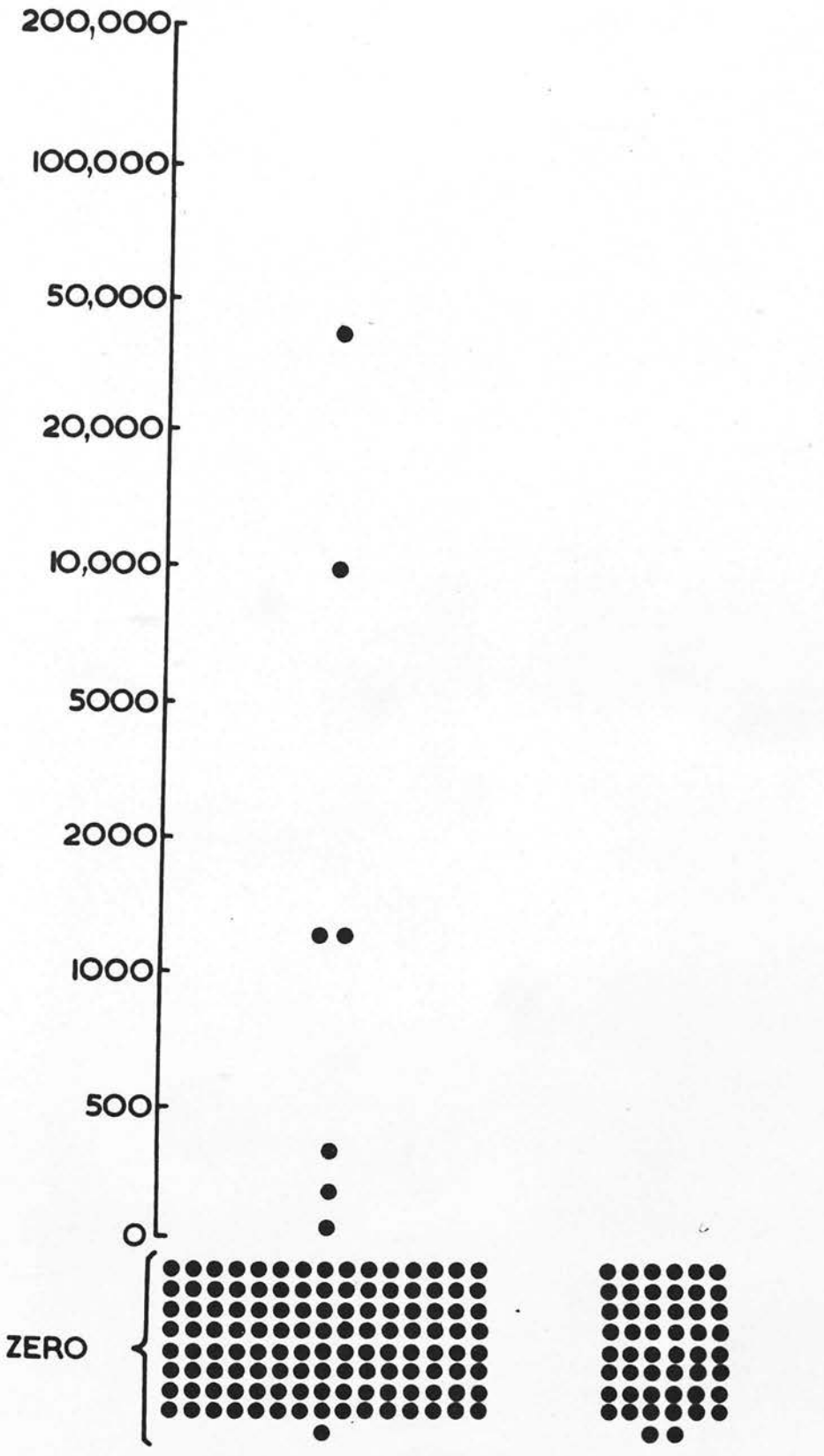
Table IV : T.C.H.

Thyrotoxicosis	Non toxic nodular goitre.
Total number of cases 74	34
Number of positive 34	4

TANNED CELL HAEMAGGLUTINATION TEST

CONTROLS

HOSPITAL POPULATION BLOOD DONORS

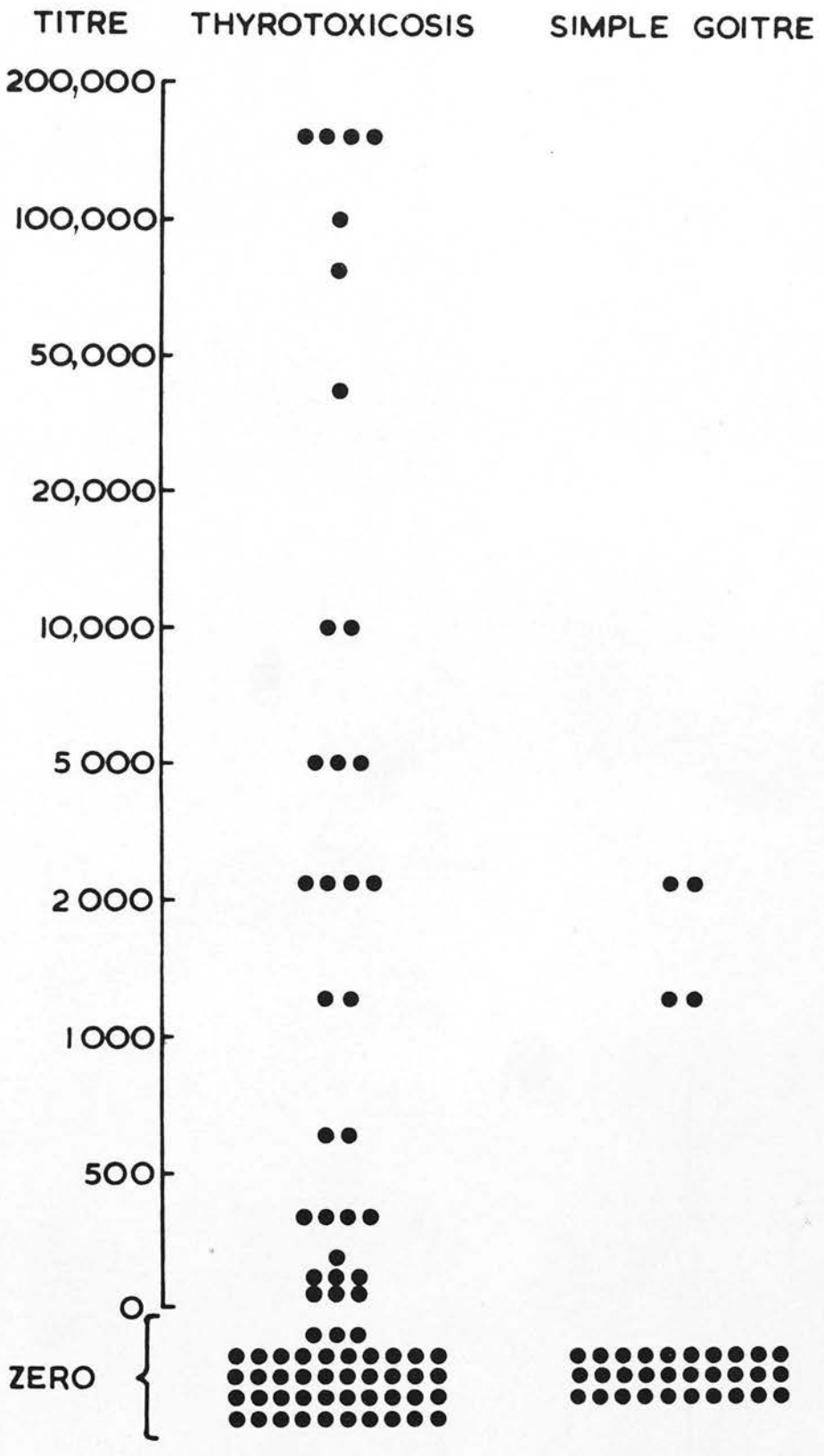


KEY: TITRE = Reciprocal of highest dilution of serum giving a positive reaction.

● = One patient

Fig. 47.

TANNED CELL HAEMAGGLUTINATION TEST



KEY: TITRE = Reciprocal of highest dilution of serum giving a positive reaction.

● = One patient

Table V : Precipitin Test.

Thyrotoxicosis	Non toxic nodular goitre
Total number of cases 74	34
Number of positive 2	0

SECTION II.

Relation of Histology to Immunology.

Thyrotoxicosis:

Material: 8 glands from patients with high antibody titres and 10 glands from patients with zero titres were examined. At least four blocks were taken from each gland.

The reason for choosing for comparison, glands with high titres, is simply that they might be expected to show more clearly the related changes than glands with more modest titres. This is indeed the case and the differences described are appreciated only if the "extremes" are studied.

Methods: The tissue was fixed in formol corrosive and routine paraffin sections prepared. The sections were stained with haematoxylin and eosin. P.A.S. and a silver impregnation method

(Slidders et al, 1958).

The sections were examined without knowledge of the antibody titre and were graded on the degree of epithelial hyperplasia, lymphoid infiltration and basement membrane damage.

Results:

Key: +++ very marked; ++ marked; + moderate; - nil.

GLANDS WITH HIGH TITRE.

	Hyperplasia	Lymphoid infiltration.	Basement membrane damage.
Case 1.	+++	++	+++
Case 2.	+++	++	+++
Case 3.	++	++	++
Case 4.	++	+++	+
Case 5.	+	++	+
Case 6.	++	-	-
Case 7.	++	++	++
Case 8.	++	++	++

GLANDS WITH ZERO TITRE.

	Hyperplasia	lymphoid infiltration.	Basement membrane damage.
Case 9.	+++	-	-
Case 10.	+++	+	-
Case 11.	+++	-	-
Case 12.	++	-	-
Case 13.	++	++	++
Case 14.	++	-	-
Case 15.	++	-	-
Case 16.	++	-	-
Case 17.	++	++	++
Case 18.	++	-	-
Case 19.	++	-	-

Non-toxic nodular goitre: Only two glands with positive antibody results were studied and two with zero titres were used as control. The methods were the same as before.

GLANDS WITH HIGH TITRE.

	Hyperplasia	lymphoid infiltration.	Basement membrane damage.
Case 20.	Focal	++	++
Case 21.	-	+++	+

GLANDS WITH ZERO TITRE.

	Hyperplasia	lymphoid infiltration.	Basement membrane damage.
Case 22.	-	-	-
Case 23.	-	-	-

Discussion:

1. Immunology.

Control sera: 8 positive results were obtained with the tanned cell test out of 198 patients. It is noteworthy that all of these atypical results were found in the hospital population and not in the healthy blood donors. Two of these eight patients had extremely high antibody titres and it turned out that one (titre 10,000) had previously been treated for thyrotoxicosis. The other case (titre 50,000) was suffering from disseminated breast cancer and showed no signs of thyroid disease. It is impossible to explain these unexpected positive results and they are not due to faulty serologic technique. On the contrary it is reasonable to assume that these patients do in fact have clinically undetected thyroid disease.

Thyrotoxicosis and nodular goitre: There is a striking difference in the incidence of antibody production in these two conditions. Antibodies were found in 46% of patients with thyrotoxicosis as compared with 12% of patients with simple nodular goitre. Furthermore the height of the titres was low in simple goitre, whereas high titres were observed frequently in thyrotoxicosis and indeed

on two occasions positive precipitin tests were present. This finding is in good agreement with Goudie, Anderson et al, who noted positive precipitin tests in 3 of 120 cases of thyrotoxicosis. It is,of course, almost certain that many more of their cases contained antibody and they did in fact demonstrate a higher incidence when they used the complement fixation test which nevertheless is not as sensitive or reliable as the tanned cell method. Similar antibodies were described by Paine, Terplan, Rose, Witebsky and Egan (1957) in one out of 19 cases of simple nodular goitre and one out of 4 cases of toxic goitre. These authors make the interesting point that the appearance of antibodies in the serum of patients, who have been subjected to thyroid surgery, may be due to the surgical trauma. Unchanged colloid acting as antigen might have found its way into the circulating blood. This does not apply to our material since the serum was collected before operation. We have, however, indirect evidence that colloid is released into the blood at operation. One patient (M.S.) had a venous sample of blood taken from her arm at the beginning of operation and a further sample from a thyroid vein at the end of the thyroidectomy. The difference in antibody titre between these two samples was

striking; the first serum gave a titre of 320 and the thyroid vein sample gave a zero result. The diminished antibody titre in the latter sample is probably due to mopping up of the antibody by antigens expelled into the veins at operation - in short an in vivo inhibition test.

In the past many have claimed an association between the nodular goitre, thyrotoxicosis and lymphadenoid goitre (Warthin, 1226; Eason, 1928; Polowe, 1934; Boyden et al, 1935; Dunhill, 1937; Vaux, 1938; Eden and Trotter, 1942; Levitt, 1954). The well known fact that a lymphoid infiltration either focal or diffuse was seen occasionally in simple goitre and more frequently in thyrotoxicosis suggested to these authors that further lymphoid hyperplasia would result in lymphadenoid goitre.

Without exception these authors ignore the striking epithelial changes seen in lymphadenoid goitre (see Chapter I) which clearly distinguish it from the hyperplastic,but normal epithelium of thyrotoxicosis.

Accordingly, one is reluctant to accept the hypothesis of thyrotoxicosis as a precursor of lymphadenoid goitre.

The finding of thyroid antibodies both in thyrotoxicosis and lymphadenoid might appear as though a connecting link between the two diseases had finally been established. Before accepting this tempting assumption one must be quite clear about the relationship, if any, between the histological appearances in the excised glands and the antibody titre.

The four simple nodular goitres examined showed prominent foci of lymphoid infiltration and basement membrane damage in two but not in the remainder. Both of these two glands were associated with a moderate titre of antibody.

The thyrotoxic glands showed a rather similar relationship. 7 of 8 glands with high titre had a moderate to marked degree of lymphoid infiltration, whereas eight of 11 glands with zero titres showed no such change. Therefore, one is entitled to conclude that a clear relationship exists between lymphoid infiltration, basement membrane damage and the presence of antibodies. Exceptions to these rules were met, and in these instances antibody is probably fixed to the tissue and is not present in the circulation.

The histological features characteristic of glands with high antibody titres were foci of "thyroiditis" (fig.47,48), plasma cell infiltration (fig. 49), Askanazy cell change (fig. 50) and basement membrane damage. These findings were also found in a very minor degree in some glands with zero titres but the important point is that they are prominent and widespread in glands with high titres. The essential requirement for antibody formation is the extravasation of colloid into the interstitial tissue. Colloid is an irritant (Ferguson, 1937) and accordingly produces an inflammatory cell reaction. The fact that antibodies are present in both thyrotoxicosis and lymphadenoid goitre does not necessarily imply a relationship between the two disorders but does mean that in both diseases colloid is liberated in an antigenic form. We remain entirely ignorant of the mechanisms of extra-follicular colloid release and there is no evidence whatsoever that the pathogenesis is the same in lymphadenoid goitre and thyrotoxicosis.

So far it has not been decided which abnormal histological feature is characteristic of antibody formation. It would seem the presence of plasma

cells is essential but an even earlier change, viz. basement membrane damage, is described in chapter V.

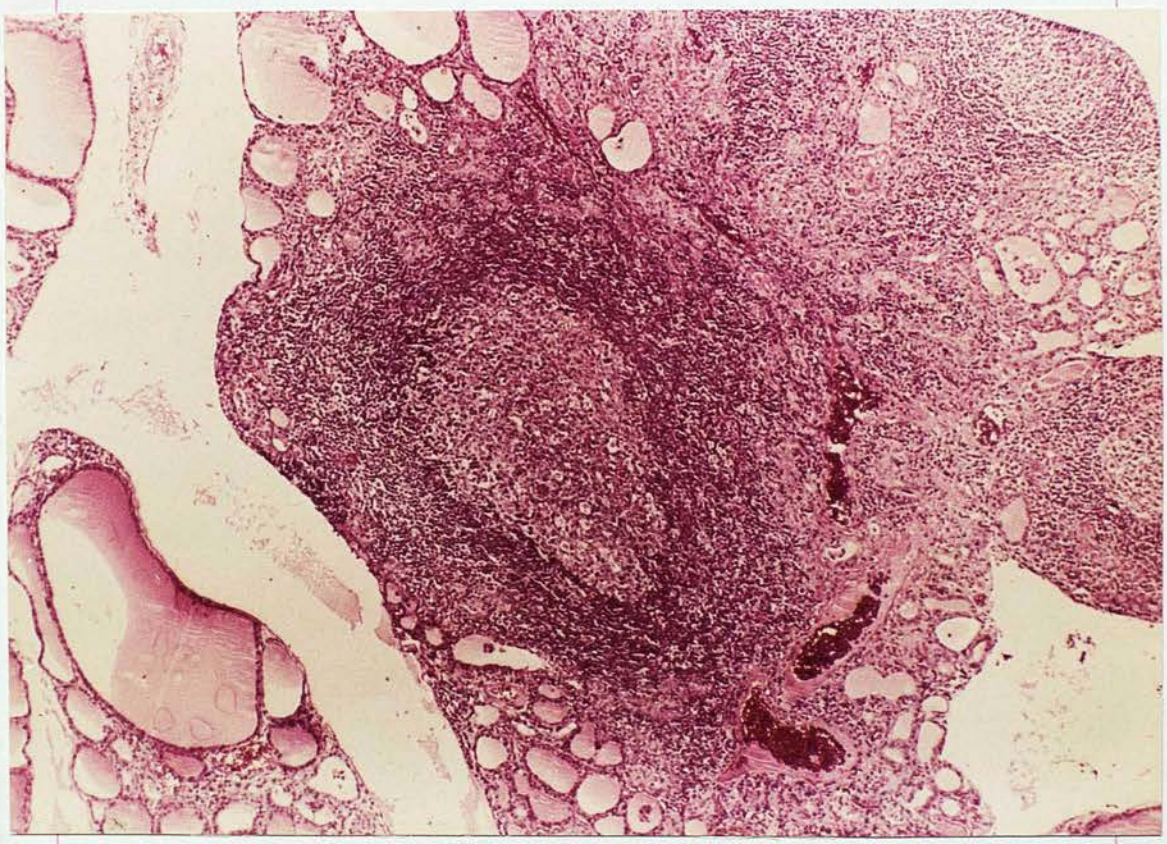


Fig. 47. (H.E. x 70).

Lymphoid follicle in thyrotoxic gland. (high antibody titre).

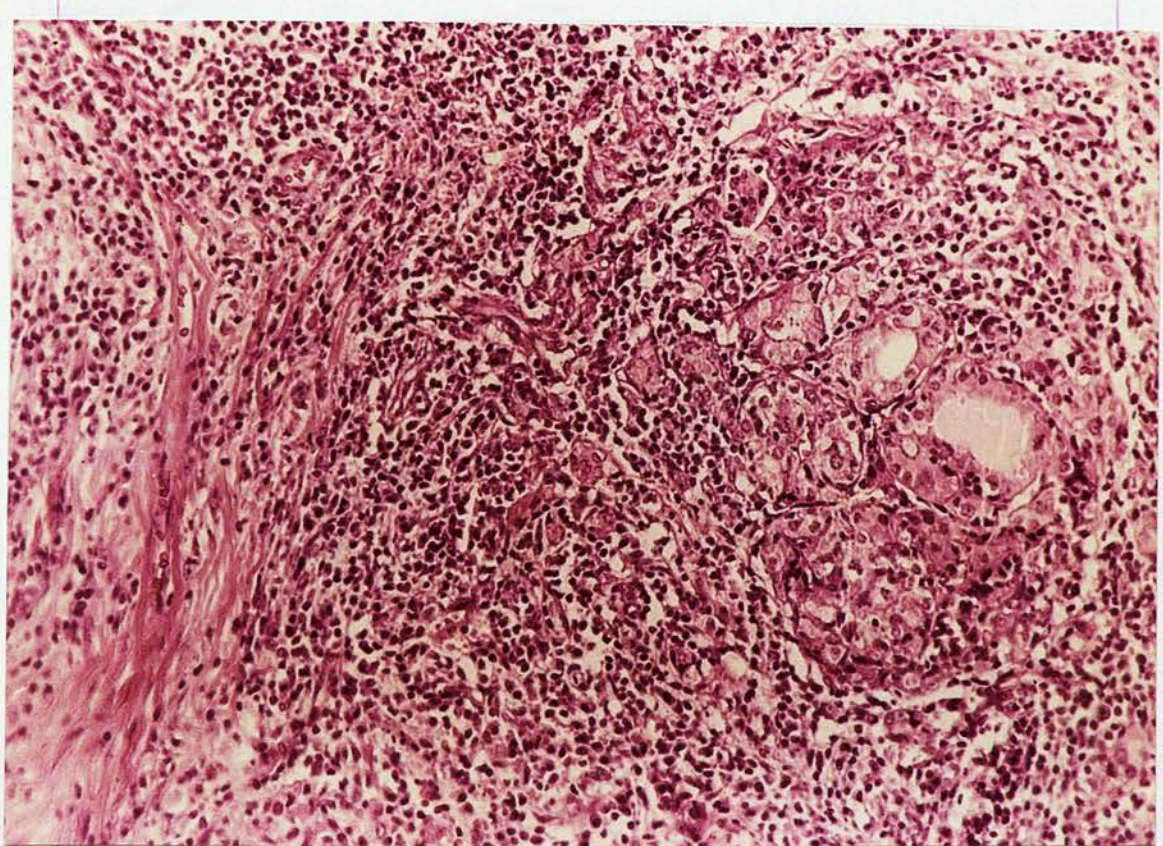


Fig. 48 . (H.E. x 200).

Focus of "chronic thyroiditis" in thyrotoxic gland (high antibody titre).

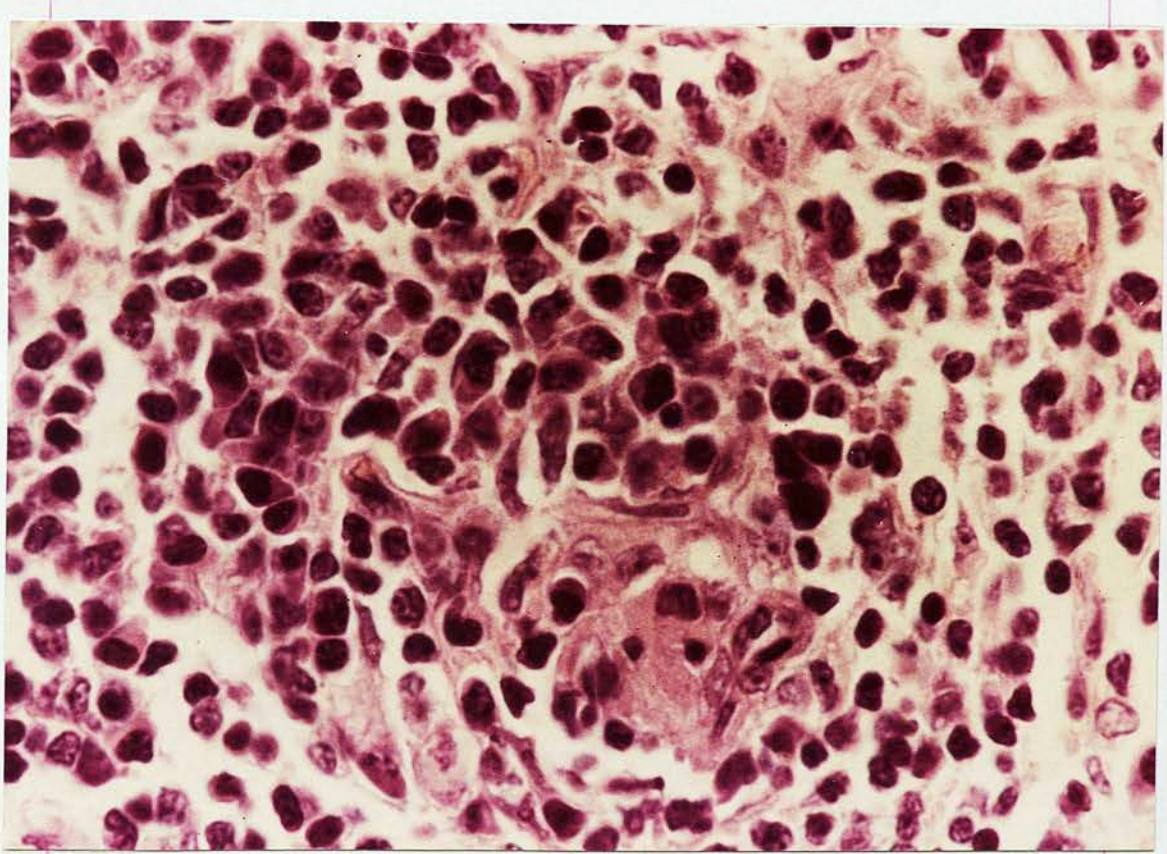


Fig. 49 . (H.E. x 1000).

Numerous plasma cells in thyrotoxic gland (high antibody titre).

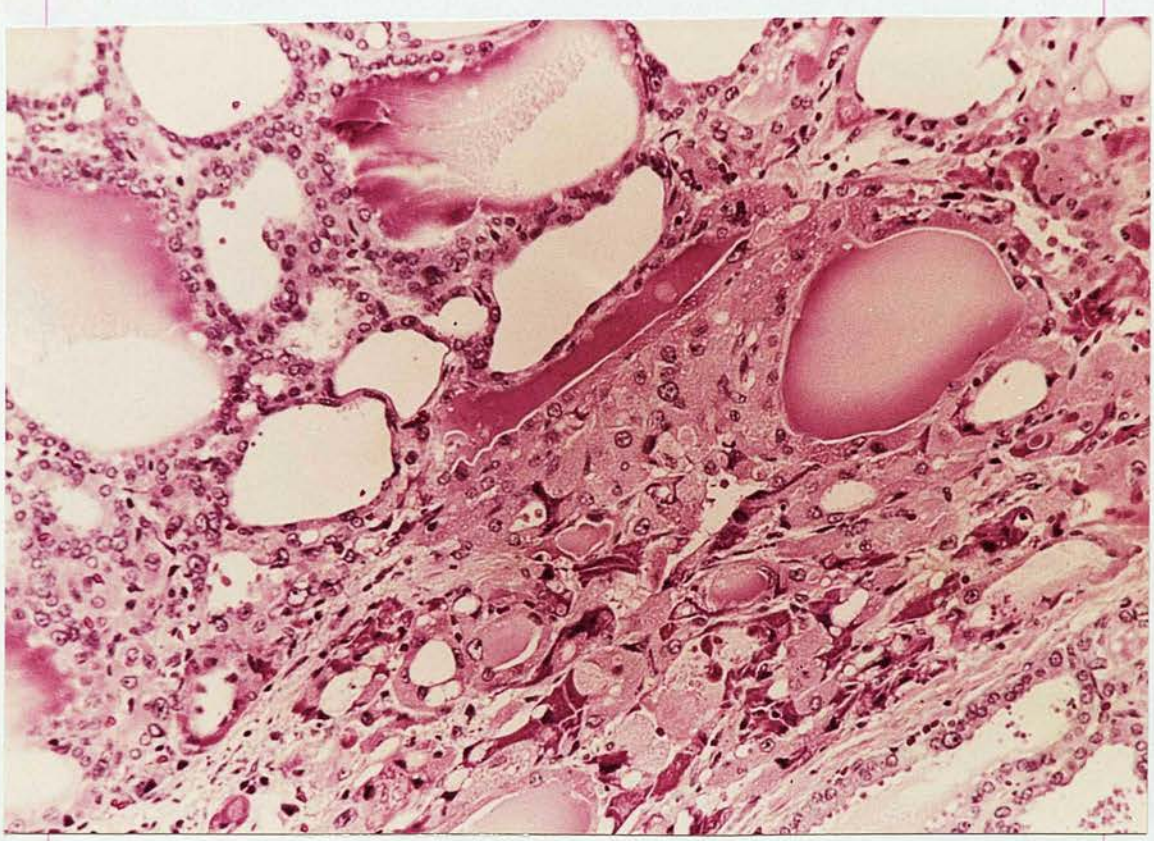


Fig. 50 . (H.E. x 200).

Askanazy cell change in thyrotoxic gland.

Chapter IV. (Section I).

ACUTE OR SUBACUTE THYROIDITIS.

Synonyms:

De Quervain's thyroiditis, Giordanengo's thyroiditis, giant cell thyroiditis, non-bacterial thyroiditis, struma granulomatosa, pseudo-tuberculosis.

Introduction:

De Quervain of Berne first drew attention to this condition in 1904 and later published an important paper with Giordanengo of Milan in 1936 describing this new form of thyroiditis. They regarded it as a distinct clinical entity which was entirely different from Riedel's and Hashimoto's strumas. The essential clinical features of the disease were pain and swelling of the thyroid gland, fever, a raised blood sedimentation rate and in some instances a history of a previous influenza-like illness. They remarked that their group of eight cases were held together by a distinctive histological picture characterised by numerous foreign body giant cells, and a variable degree of fibrosis. The fibrosis in some of their cases was quite marked and they discussed the relationship of subacute thyroiditis to Riedel's disease in the following terms.

"The observations at operation show that at times the affected tissue is as firm as in Riedel's illness and that it crunches under the knife as in Riedel's illness. The adhesion of the affected lobe with the surrounding tissue is, to be sure, as we have observed ourselves, a comparatively pronounced one, but the gland is not adhered into a solid mass with the neighbouring tissues to the same degree as is depicted in most descriptions of "Riedel".

The present state of our knowledge can be summarised roughly in the following way; if one wants to designate every occurrence in the thyroid gland of a taut connective tissue,which is as solid as cartilage,as Riedel's illness, then one can say that individual cases of simple subacute or chronic thyroiditis with giant cells lead to "Riedel." If one takes into account the whole clinical picture of both illnesses together with their histological characteristics one will interpret thyroiditis connected with the formation of foreign body giant cells for the present as a disease picture on its own.

Thyroiditis associated with the formation of giant cells can lead in the subacute and chronic phase to a solid growth of connective tissue

which reminds one of Riedel's thyroiditis. The clinical course of both illnesses is, however, a different one in typical cases. It will be a question for the future to ascertain whether a particular histological type corresponds to the clinical notes of Riedel's illness or whether this conception is to be used simply as a collective term for the solid fibrous degeneration in illnesses of the thyroid family varying in origin and histological character."

Despite this clear and detailed report on subacute thyroiditis the condition has frequently been confused in the literature with Riedel's disease. This confusion is partly due to lack of acquaintance with De Quervain's writings and partly to the resemblance of the disease to the popular and erroneous concept of Riedel's thyroiditis. Such an instance is seen in McKnight's (1936) exemplary report on "Riedel's thyroiditis" where the author's lucid description makes it quite clear that he is in fact dealing with subacute thyroiditis. Clark (1958) found ten similar examples in the literature.

The disease was recognised by Schilling (1945)

who reported three cases but preferred to call them giant cell variants of struma fibrosa.

Lindsay and Dailey (1954) made a clinical and pathological study of 37 patients. They were able to find only 23 patients with this disease out of 7,263 thyroidectomies at the University of California Hospital and the condition appears to be rare. These authors made the interesting point that subacute thyroiditis was the only thyroid disease in which existed the paradox of an elevated serum protein bound iodine and diminished uptake of radio-active iodine by the gland. They believed that lesions might be accounted for by hypersensitivity and although they discussed the possibility of viral injury they found no adequate evidence to support this view. However, Eylan et al (1957) reported an outbreak of subacute thyroiditis in Israel during a mumps epidemic and were able to demonstrate a high titre of mumps complement fixing antibody in ten of their eleven cases. This was followed by a single case report by Felix-Davies (1958) where there was an association between mumps and subacute thyroiditis, and Stuart (unpublished) noted a similar case somewhat earlier this year.

Reports on subacute thyroiditis are very scanty in the British literature. Fraser and Harrison (1952) published three cases and believed the epithelial injury might result from viral invasion in view of the prodromal symptoms. Selwyn Taylor (1955) claimed to have recognised six cases over three years and suggests that increasing familiarity with the histological features will result in a greater incidence of the disease.

The present study reports briefly 7 cases and emphasises the great variability in the histological picture. The sera of six of these patients was examined for thyroid antibodies.

Pathology of Subacute Thyroiditis.

Gross appearances:

The gland is diffusely enlarged, firm and white. The external surface is smooth and the capsule thin. Minor adhesions may be present but this is not always so. (Fig. 51). On section the tissue is hard and crunches under the knife. The parenchyma is pearly white, glistening and homogeneous (Fig. 52). This diffuse affection is not always met with since the disease is sometimes focal and recurrent attacks may produce a pleomorphic picture where foci of normal tissue mingle with areas of active thyroiditis and foci of fibrosis.

Microscopic appearances:

The early stage of the disease is characterised by swelling and desquamation of the epithelium with the formation of large giant cells. The basement membrane of the follicle undergoes dissolution and colloid escapes into the interstitial tissue where it provokes an inflammatory reaction. This reaction is composed in the early stages of numerous macrophages, lymphocytes and mononuclear cells. Small foci of polymorphonuclear leucocytes - micro abscesses -

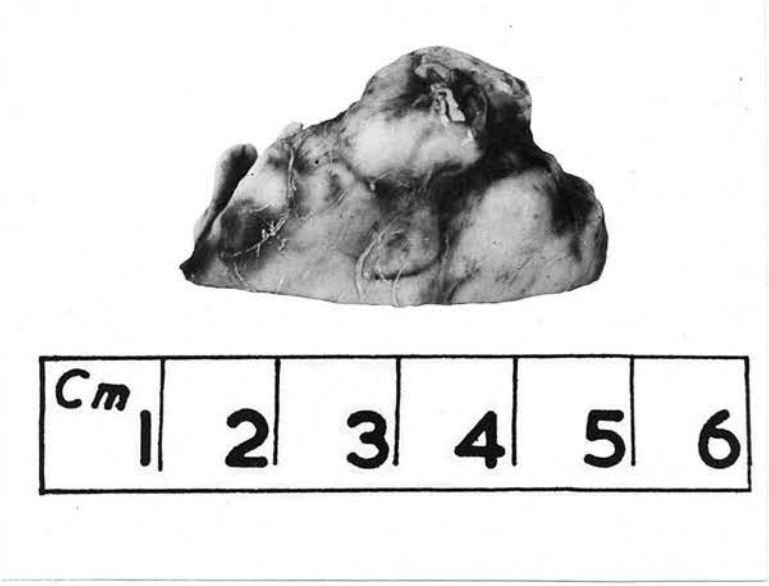


Fig. 51.

External appearances of gland in subacute thyroiditis.

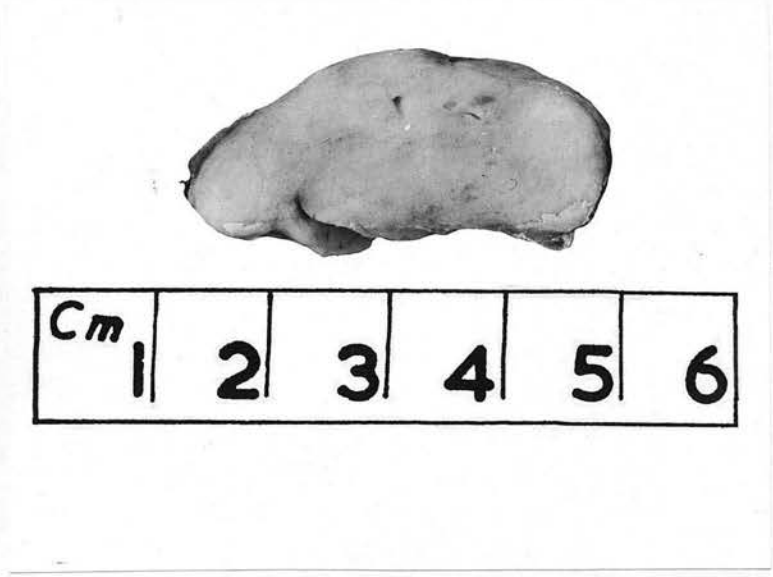


Fig. 52.

Cut surface of gland in subacute thyroiditis.

are sometimes seen but on the whole they form an insignificant and unimportant part of the picture. Later, numerous plasma cells, and fibroblasts are present. The degree of fibrosis seems related to the severity of the initial parenchymatous destruction. If this is severe marked fibrosis will result but not uncommonly one sees only an increase of interstitial connective tissue. The amount of fibrosis is sometimes greater than one would expect from the clinical duration of disease. This also has been the experience of Moran in Pittsburgh (personal communication). In the recovery phase the epithelium regenerates and forms small follicles which may be round or slit-like. The colloid is unusually pale and the epithelium is flattened or low cuboidal with prominent darkly staining nuclei. Although subacute thyroiditis is regarded as a self terminating disease ending in complete recovery it seems certain that some cases do in fact proceed to severe fibrosis.

The following cases have been selected to show the pleomorphism of the microscopic appearances and illustrate the great difficulty which attends its diagnosis when the biopsy is taken at a late stage. Several photographs from

different parts of the same specimen are shown because in no other way can a comprehensive picture be given.

Case I.

Female aged 38 years, with comparatively recent stony hard enlargement of thyroid gland. Slight discomfort in the neck but no pressure symptoms or acute pain. Serum taken from the patient 6 weeks after subtotal thyroidectomy showed a titre of 1/80 with the tanned cell haemagglutination method. The same sample of serum gave a positive complement fixation with soluble mumps antigen to a titre of 1/260 (by courtesy of Dr. R.H. Swain, Reader in Virology, University of Edinburgh). The patient has had no recent contact with mumps and there was no clinical evidence of an associated mumps infection.

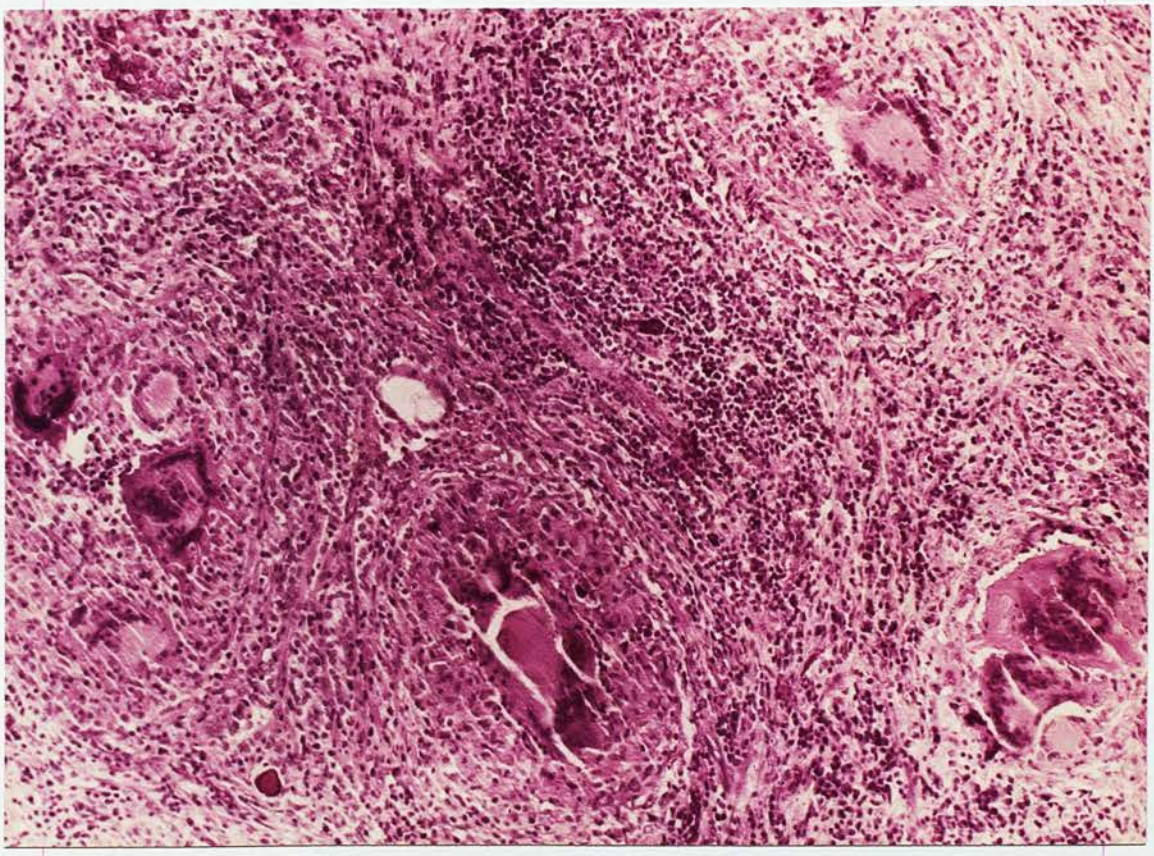


Fig. 53 . (H.E. x 120.) (Case I).

This shows complete loss of normal architecture, numerous giant cells and dense round cell infiltration.

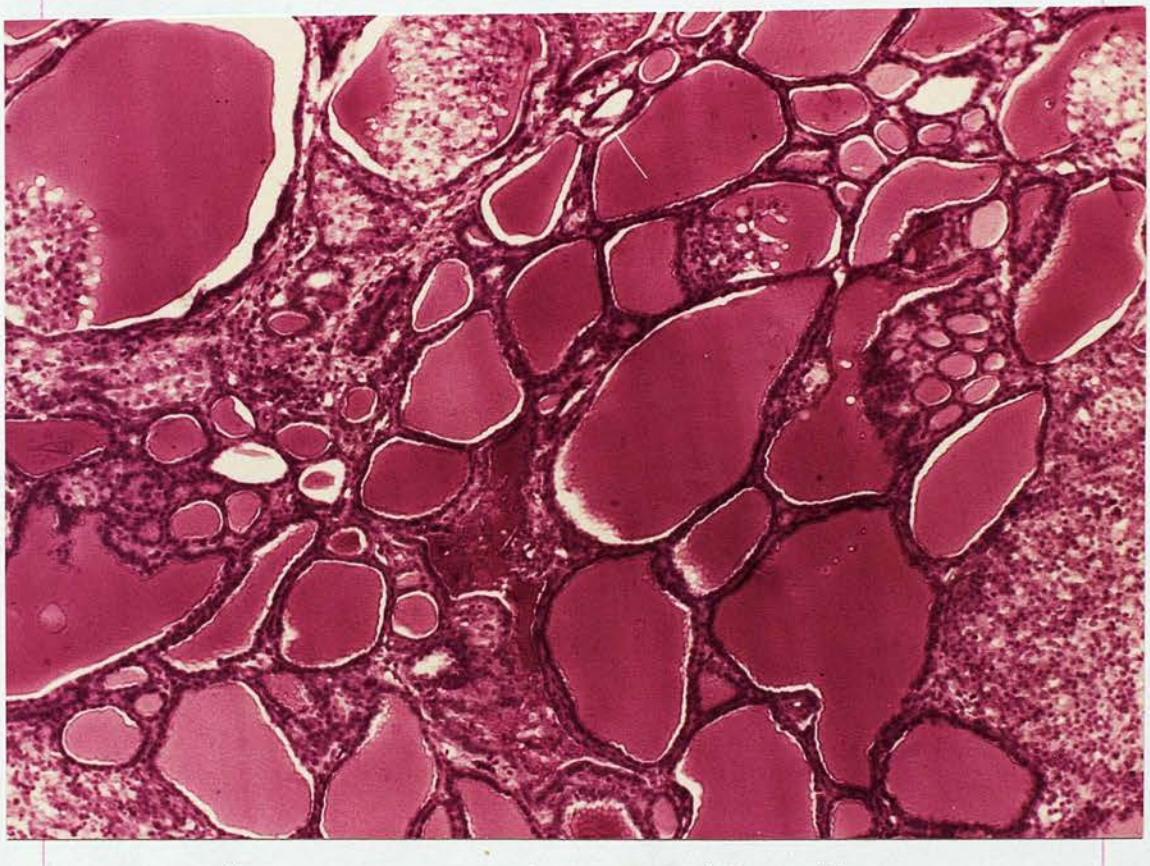


Fig. 54. (H.E. x 120) (Case I).

On the whole a normal pattern except for presence of swollen desquamated cells within follicles.

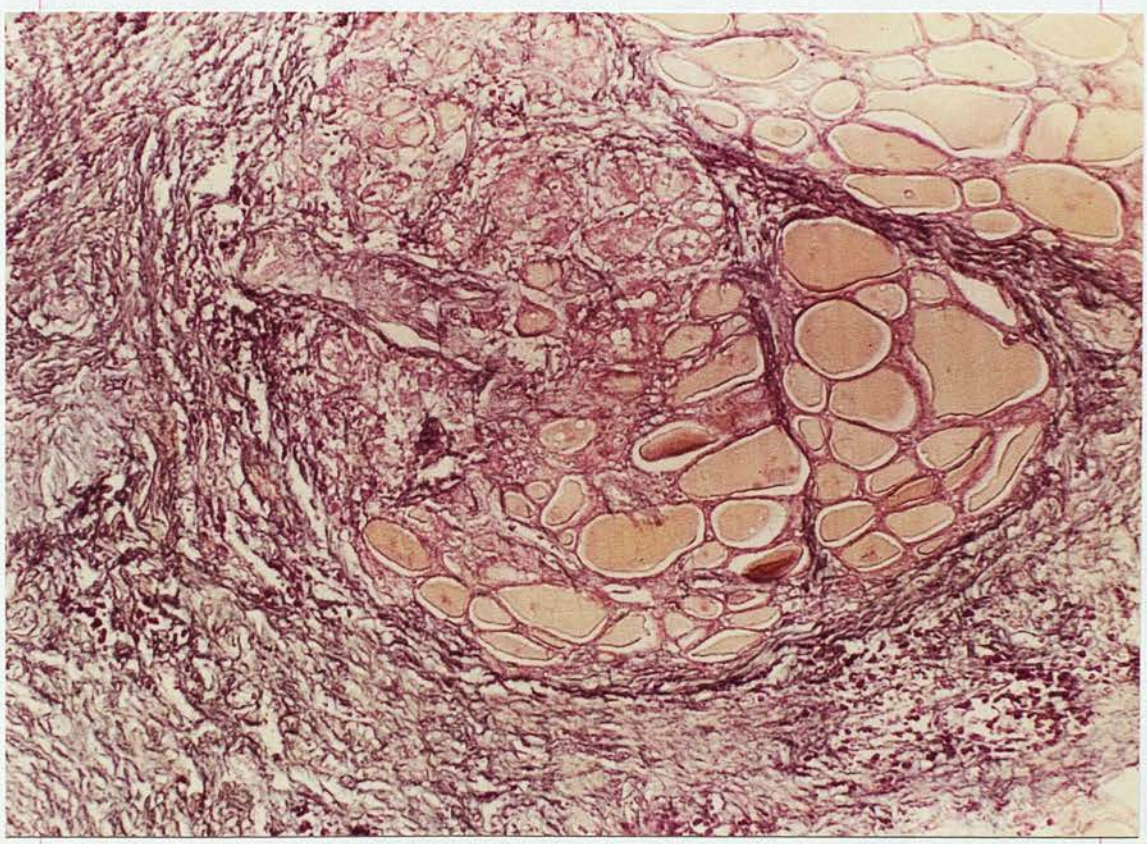
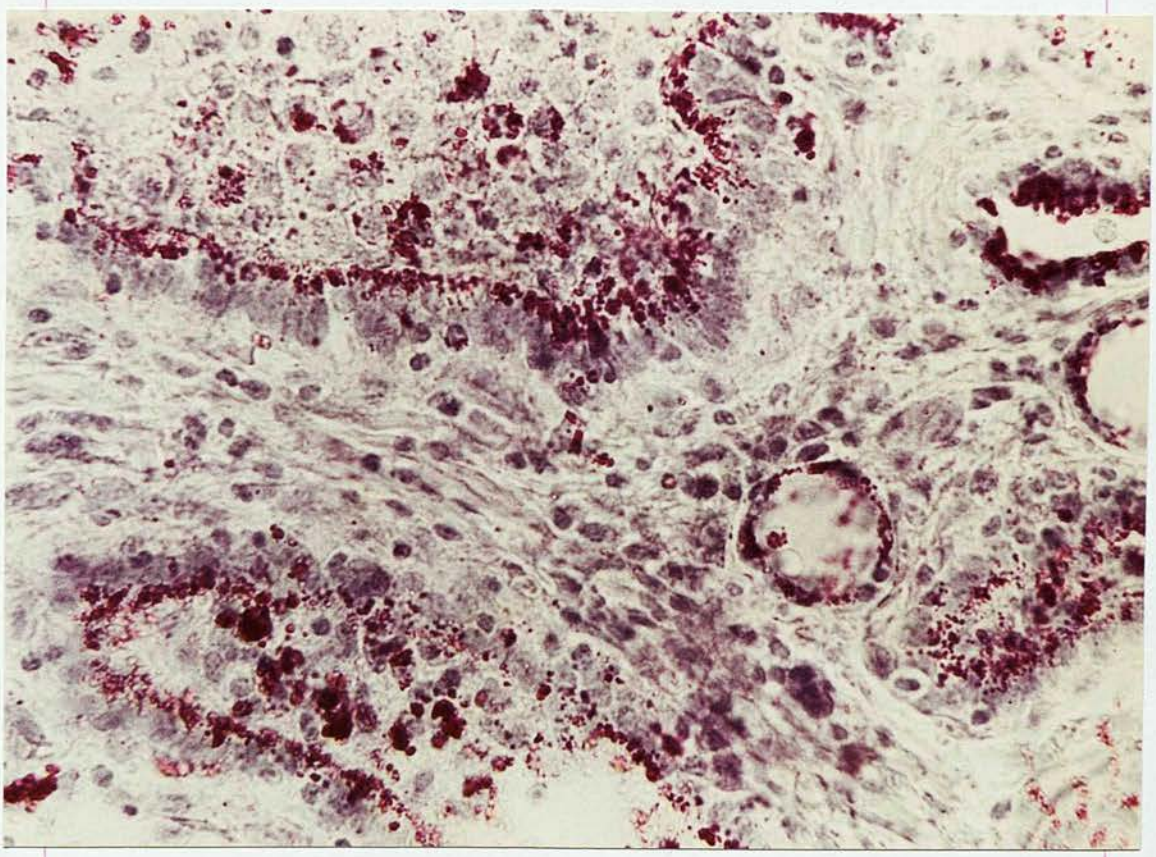


Fig. 55 . (Gallego x 170). (Case I).

Quite dense fibrosis surrounding lobule of normal parenchyma.



(Case I)

Fig. 56 . (Scharlach Red, frozen section x 400)

Marked fatty degeneration of epithelial cells.

Case II.

Female aged 45 years, suffered severe pain in the right side of the neck following an attack of influenza several weeks previously. A small tender diffuse goitre was present and the biopsy was taken 2 weeks after the onset of clinical illness. Her serum at this time containing antibodies to thyroglobulin in a titre of 1/2560 (tanned cell method) which several weeks later fell to zero.

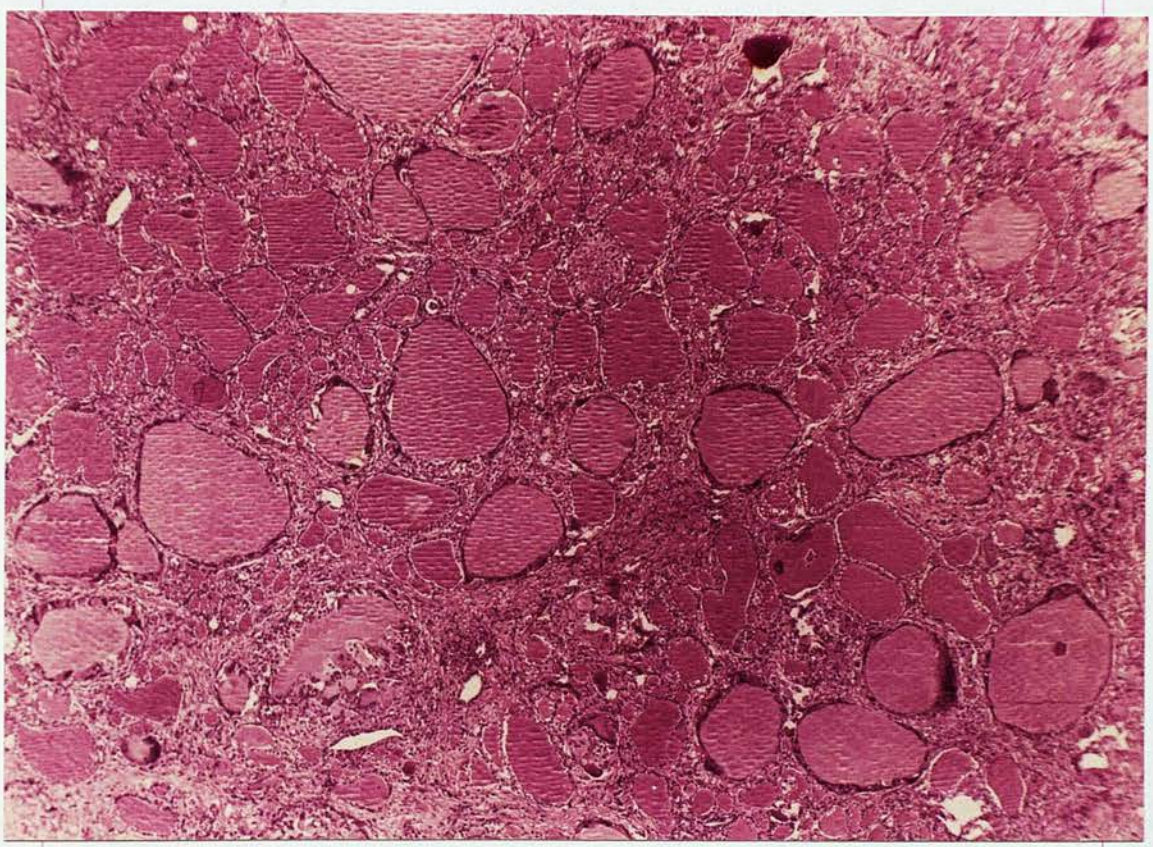


Fig. 57 . (H.E. x 50). (Case II)

This shows desquamation of swollen epithelium, interstitial round cell infiltration and segmental fusion of the follicular epithelium which has a smudged basophilic appearance. Many acini appear normal.

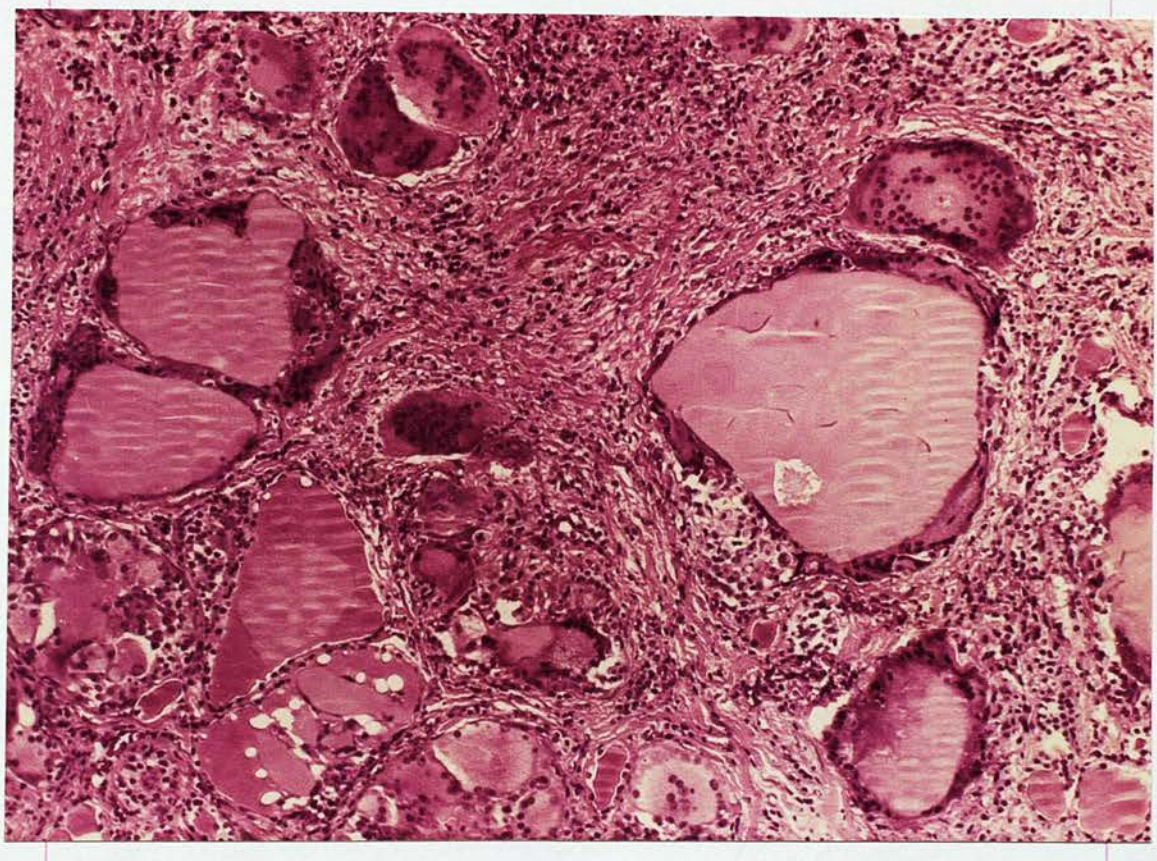


Fig. 58. (H.E. x 130). (Case II)

This illustrates the characteristic giant cells and shows an early stage in their formation, viz. syncytial fusion of the epithelium basophilia of cytoplasm and great irregularity in nuclear arrangement. The stroma is characteristic; it has a peculiar mucoid appearance and is infiltrated by lymphocytes and plasma cells.

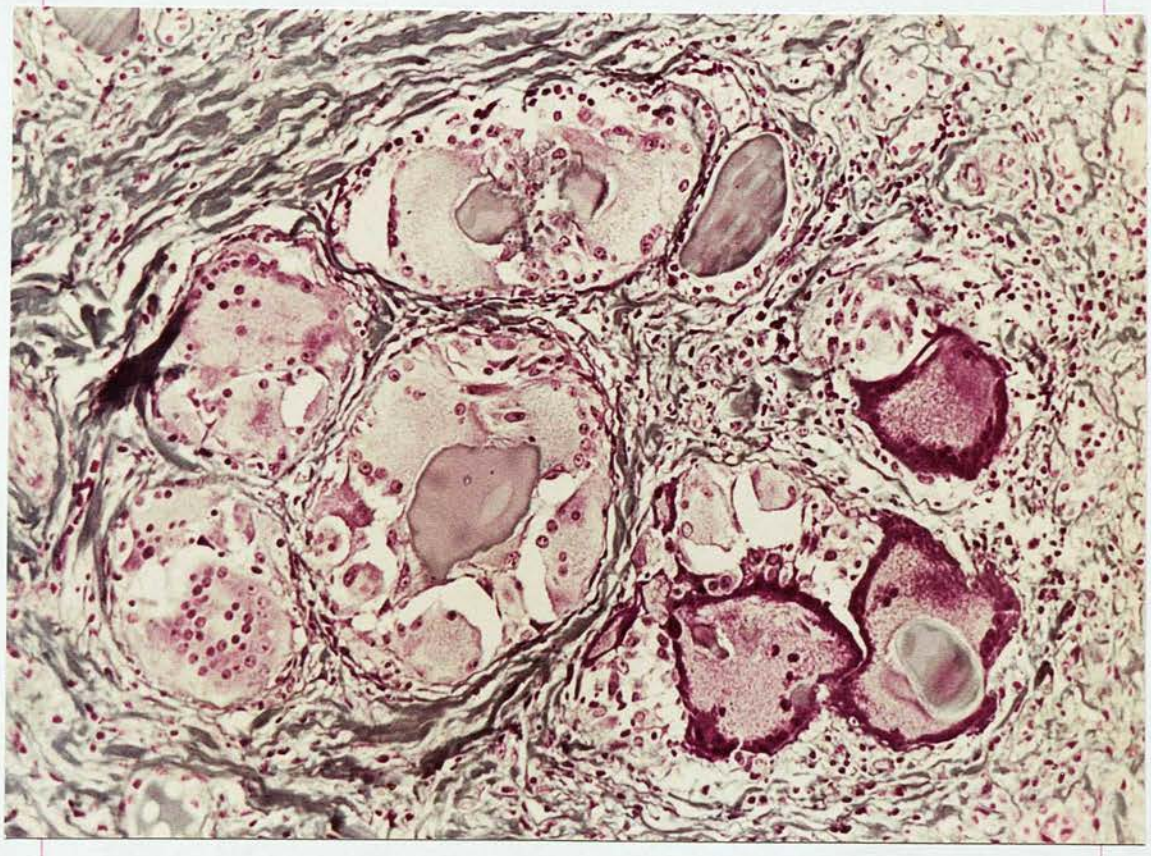


Fig. 59 . (Masson trichrome x 200.) (Case II)

Note fibrous tissue surrounding follicles. This stain reveals a purple or fuchsinophilic margin to some of the giant cells. This is an early stage in their formation and leads subsequently to the appearance seen in the four follicles on the right.

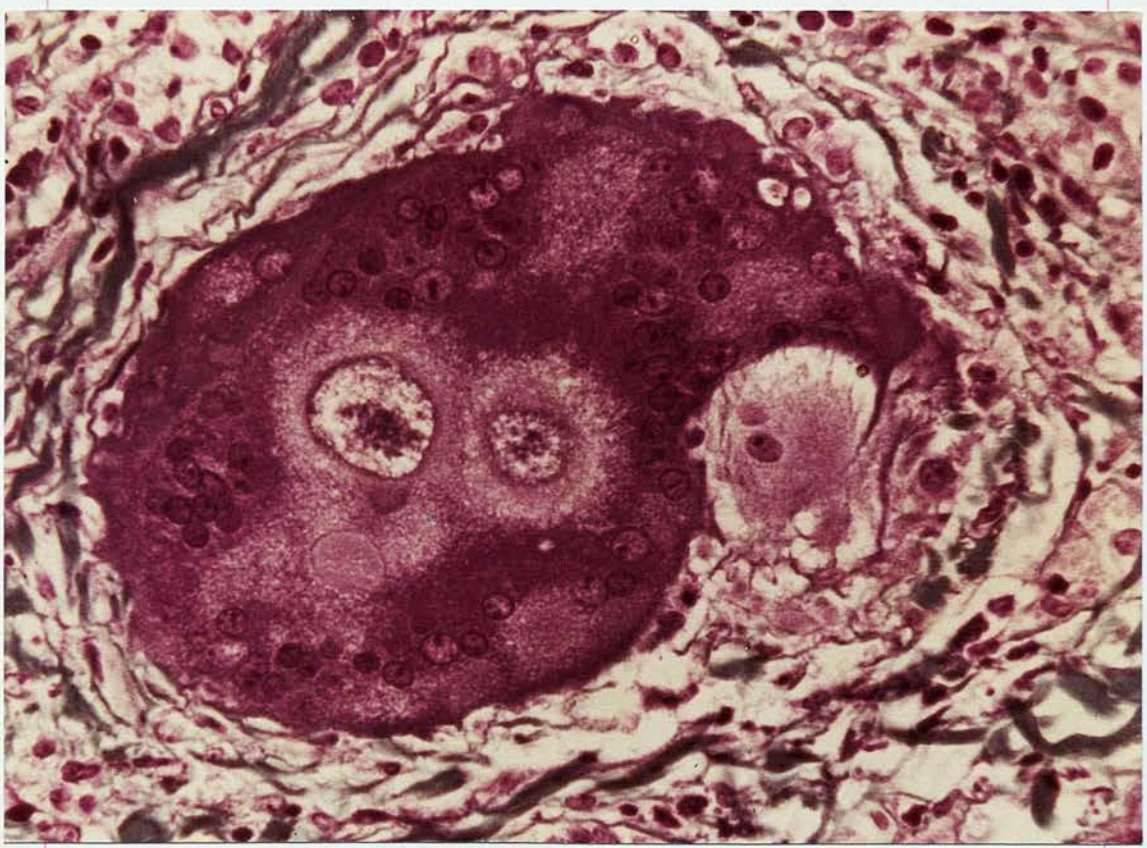


Fig. 60 . (Masson trichrome x 600). (Case II)

This shows two large swollen karyolytic nuclei in the centre of a giant cell. The nuclei at the periphery are derived from the follicular lining and several show rod shaped inclusions which resemble virus. These inclusions are not seen in normal cells.

Case III.

This patient has been conscious of a swelling in the neck for many years. For two weeks she has noticed pain and redness of the skin over the swelling and two days ago it ulcerated and discharged pus from which pneumococci were grown on culture. The infection was controlled by penicillin and a biopsy performed. The clinical diagnosis was fungating malignancy. She has made a good recovery and is in good health. A radio-active iodine test at the time of illness showed normal thyroid function and no antibodies were found in the serum.

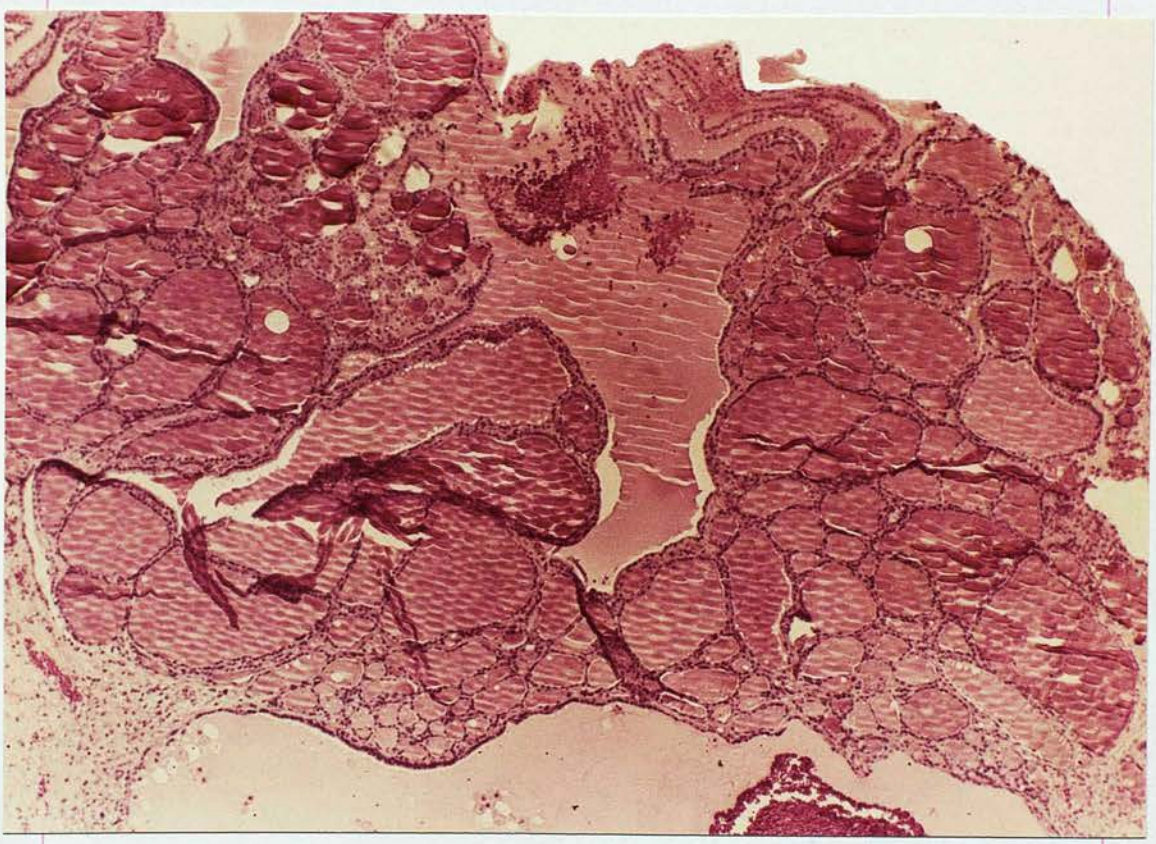


Fig. 61 . (H.E. x 70). (Case III)

Locus of normal thyroid tissue.

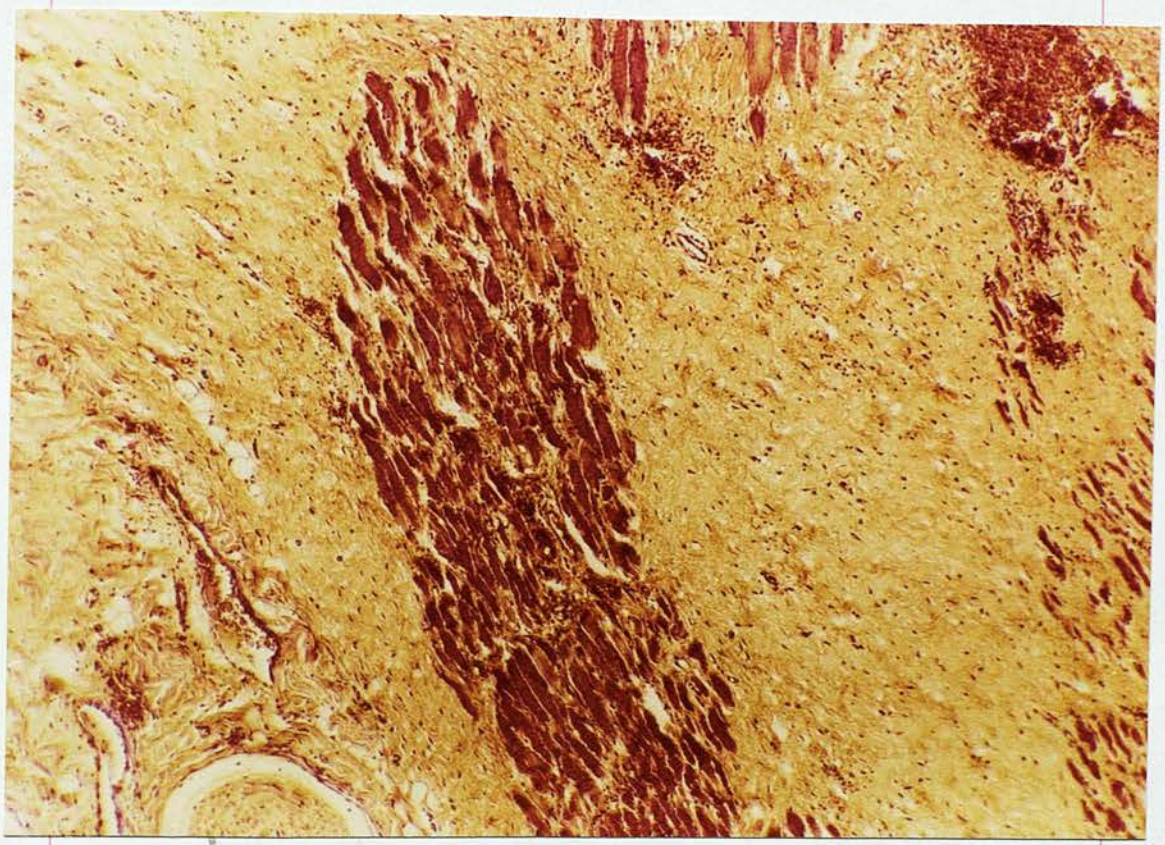


Fig. 62 . (Lissamine Tartizene x 70). (Case III)

Extra capsular adhesions showing atrophic muscle fibres (red) surrounded by connective tissue (yellow-brown). The adhesions were not dense and in no way resembled those described by Riedel. Small nerves were prominent in this section and part of one is seen at the edge of the photograph.

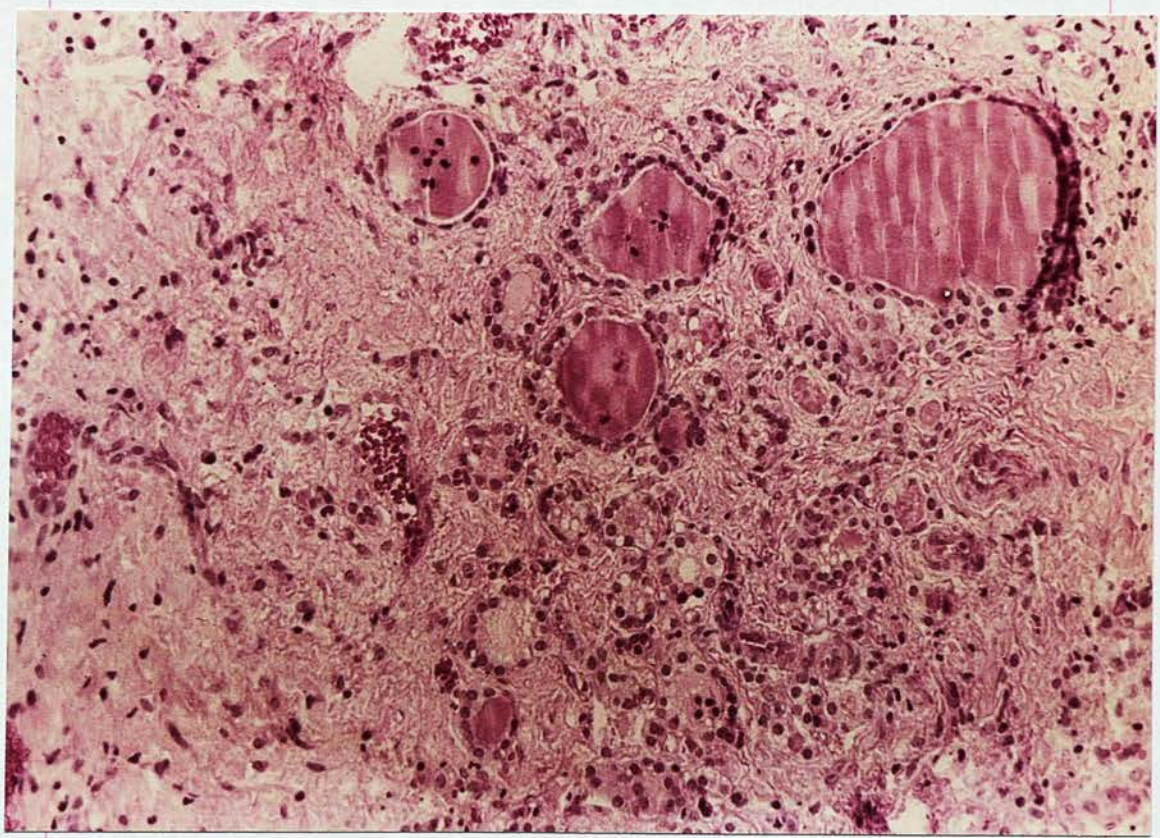


Fig. 63 . (H.E. x 200). (Case III)

The follicles are small and the interstitial tissue has a basophilic mucoid appearance. No giant cells are seen but the interstitial changes are very similar if not identical with those seen in classical subacute thyroiditis.

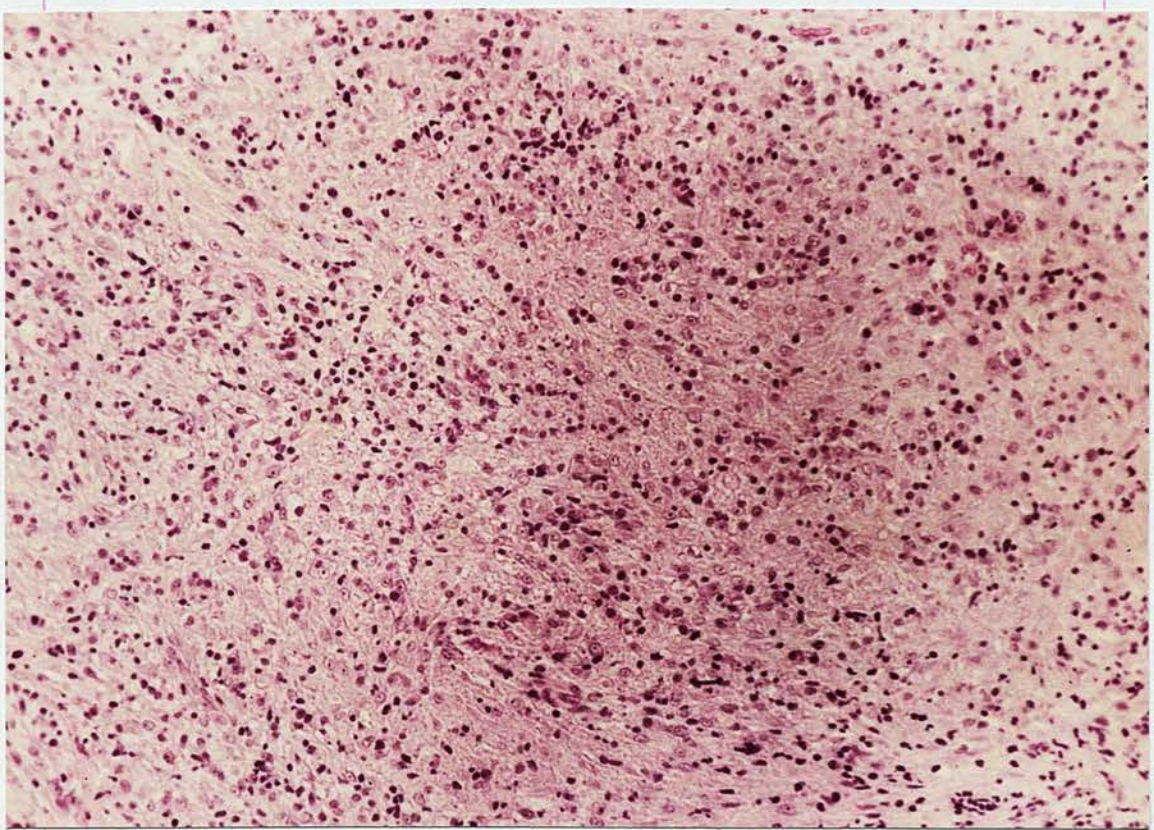


Fig. 64 . (H.E. x 200). (Case III)

This shows a typical field of the interstitial tissue. No recognisable follicles are present, and the disordered array of cells include isolated epithelial cells, lymphocytes, plasma cells, macrophages and fibroblasts. The whole has a basophilic tinge.

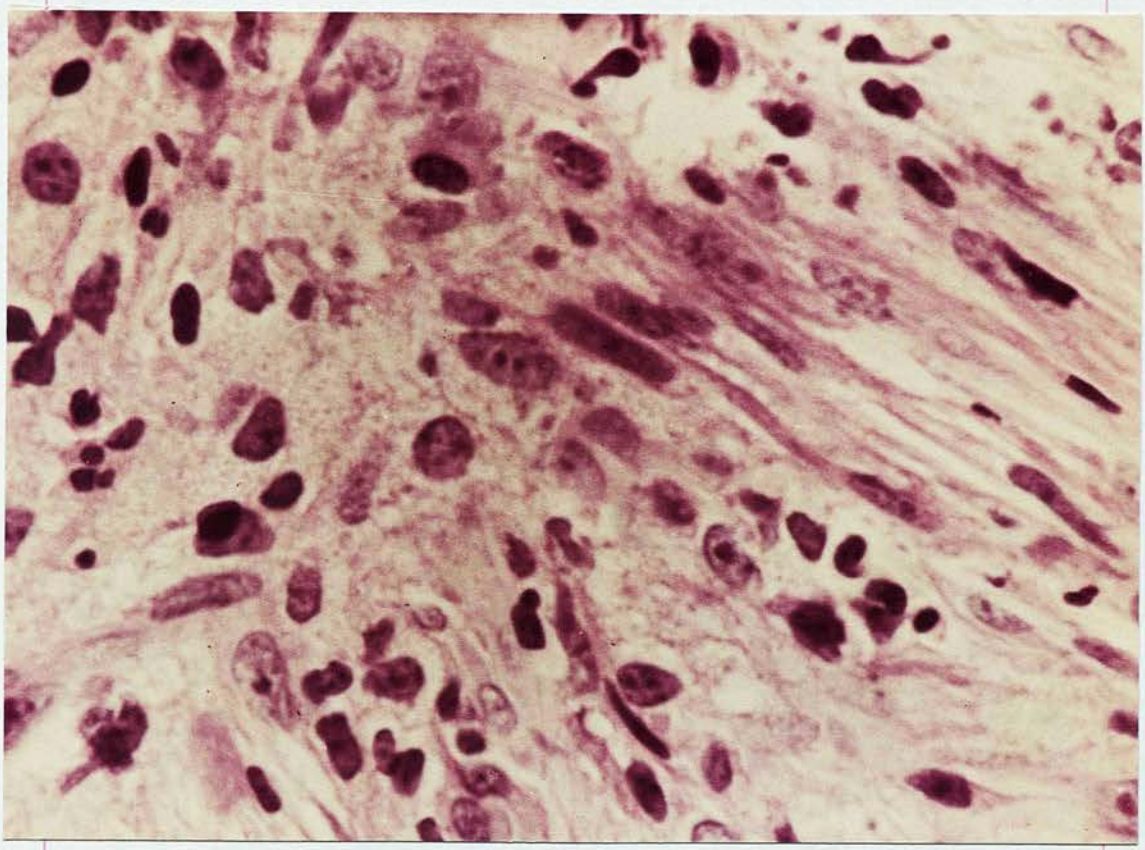


Fig. 65. (H.E. x 1000). (Case III)

High power view of fig. 64 showing spindle cells, plasma cells, lymphocytes and epithelial nuclei.

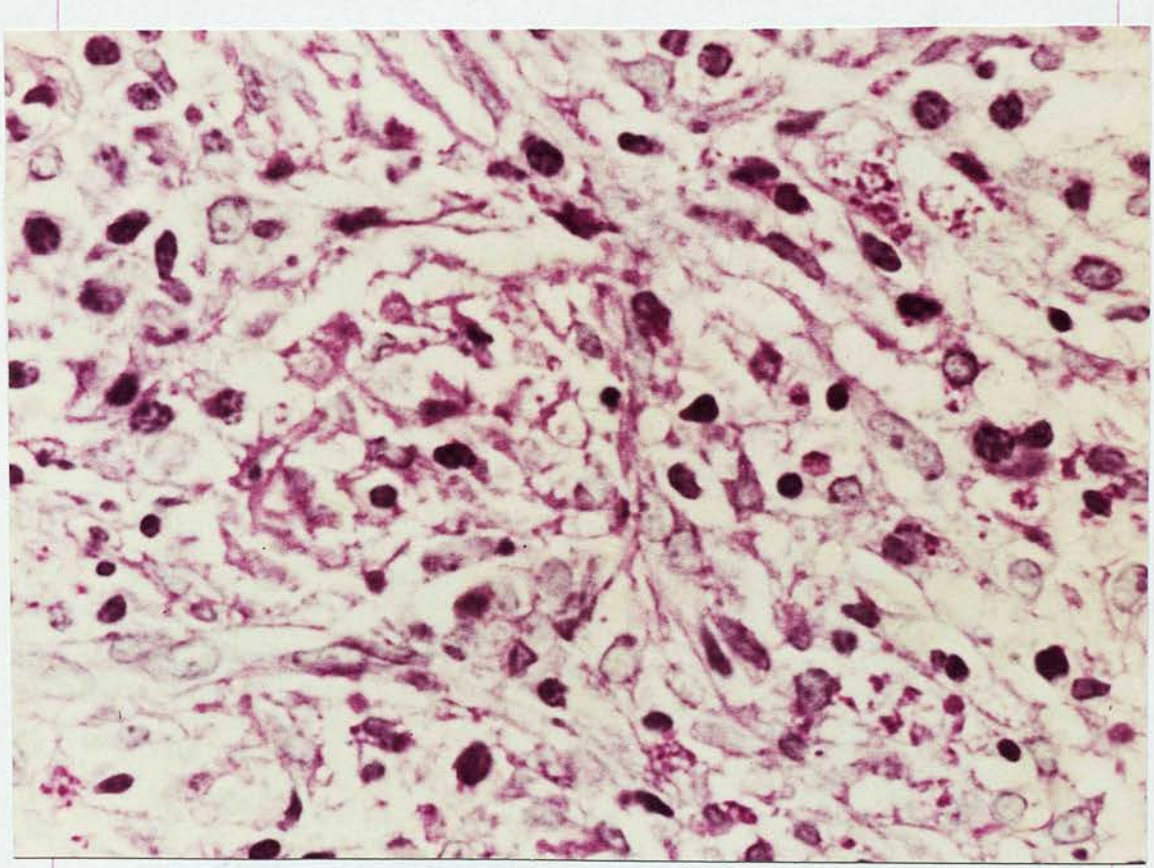


Fig. 66 . (P.A.S. x 1000.) (Case III)

This shows numerous P.A.S. positive granules in the cytoplasm of cells with ample clear cytoplasm. Colloid stains strongly with P.A.S. and although some of these cells are macrophages others are epithelial cells derived from fragmented follicles.

The presence of moderate adhesions, focal inflammatory changes and an interstitial infiltrate with many cells showing P.A.S. positive granules, is in keeping with subacute thyroiditis. The histology shows also a remarkable resemblance to Riedel's original illustrations (see Section III, Chapter IV), and it is of interest that Professor Wegelin made a microscopic diagnosis of "Riedel's struma" on the fourth case of de Quervain and Giordanengo.

No pneumococci or pus cells were seen in the sections and, despite the effect of penicillin therapy, it is difficult to believe that these appearances could be caused by a suppurative process within the thyroid. It seems more probable that a hypersensitivity reaction, as suggested by Lindsay and Dailey (1954), is perhaps the true cause.

The absence of giant cells is a formidable objection to the diagnosis of subacute thyroiditis but in view of the small size of the biopsy, one cannot make too much of this point.

Case IV.

Female aged 41. This patient gave a 6 month history of a unilateral swelling in the neck. It was painless, unusually hard and felt rather like a lymphadenoid goitre. She was myxoedematous at the time of examination when a diagnostic biopsy was taken. Serum antibody titre of 1/160,000 later falling off to zero.

Conclusion: (Case IV)

The histology is extremely difficult and clearly demonstrates the almost impossible task of correctly identifying some cases of non specific chronic thyroiditis. The epithelial changes described are believed to be consistent with acute injury. The interstitial changes are indistinguishable from those of classical De Quervain's thyroiditis. The relatively acute giant cell stage has passed and only fibrosis remains. Accordingly it seems logical to label this as a rather late stage of a progressive subacute thyroiditis.

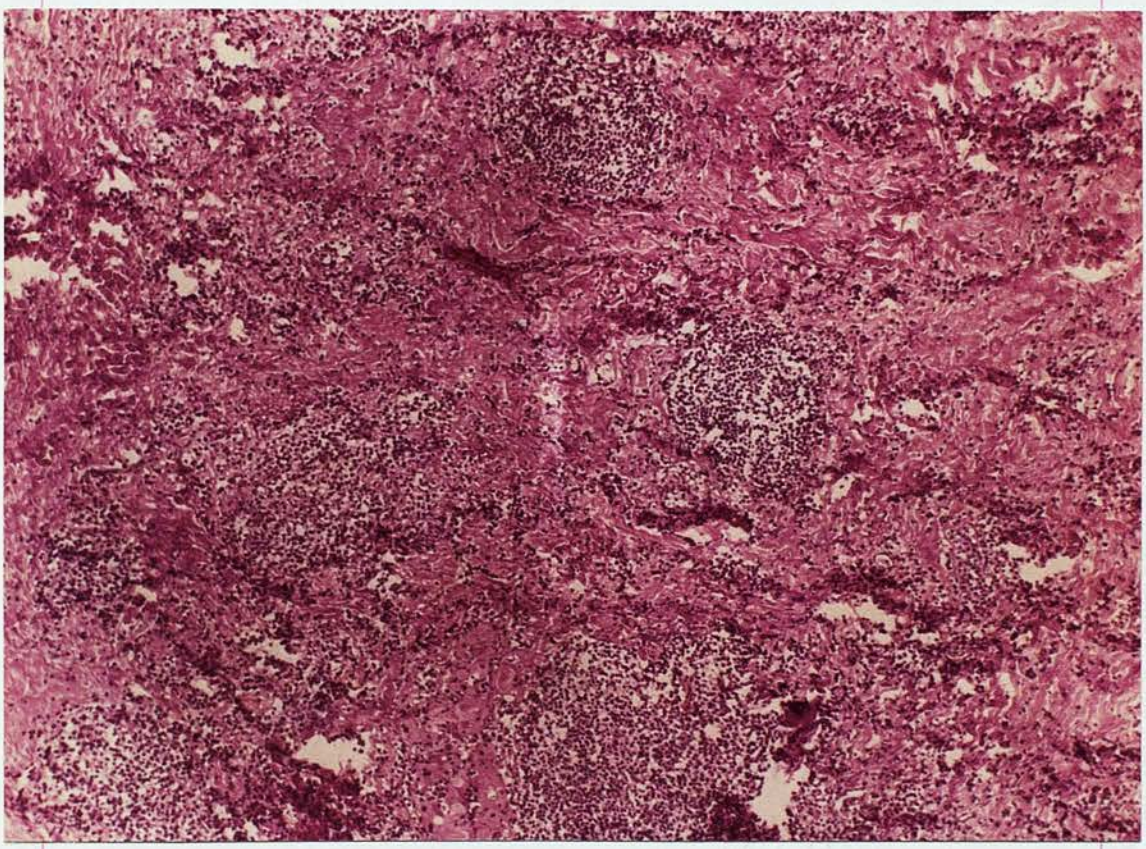


Fig. 67 . (H.E. x 70). (Case IV)

This shows the general appearance of the tissue - foci of round cells separated by dense fibrous tissue.

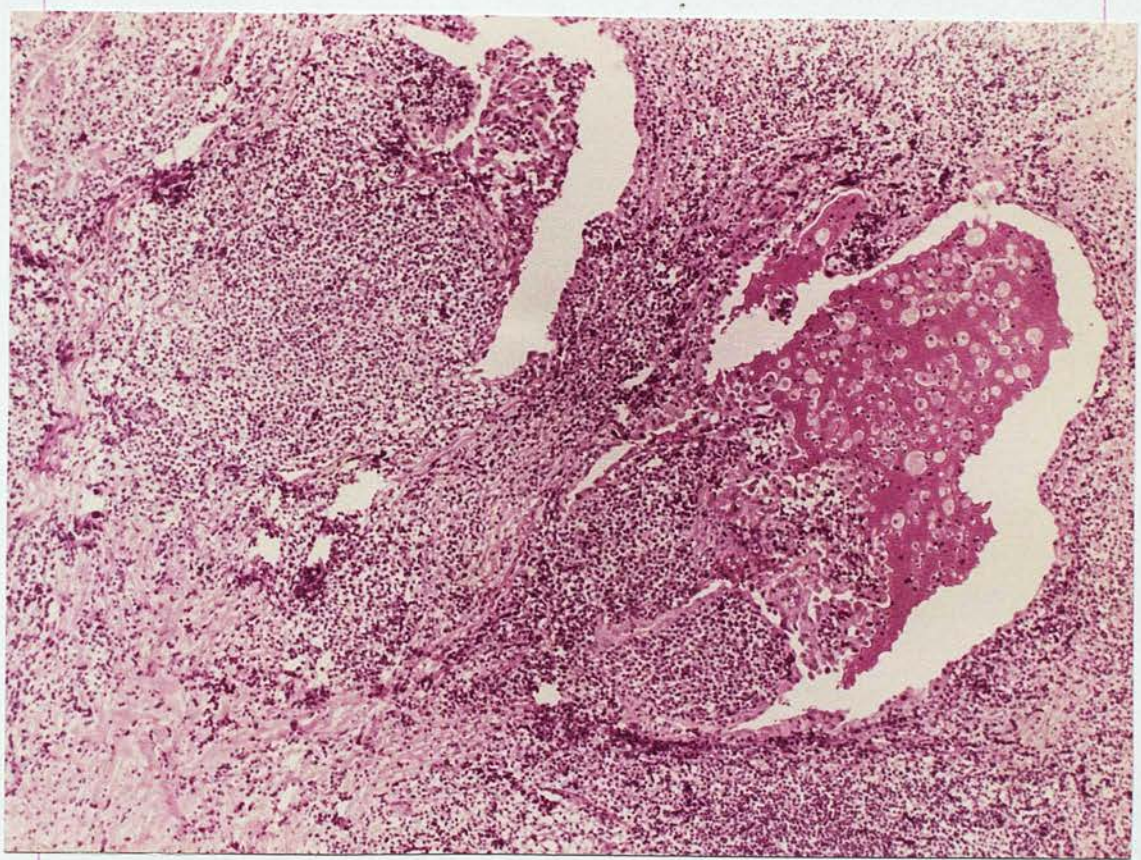


Fig. 68 . (H.E. x 70). (Case IV)

A search through the section reveals the remains of two follicles. Note the swollen desquamated epithelial cells and rather characteristic mixed type of infiltrate.

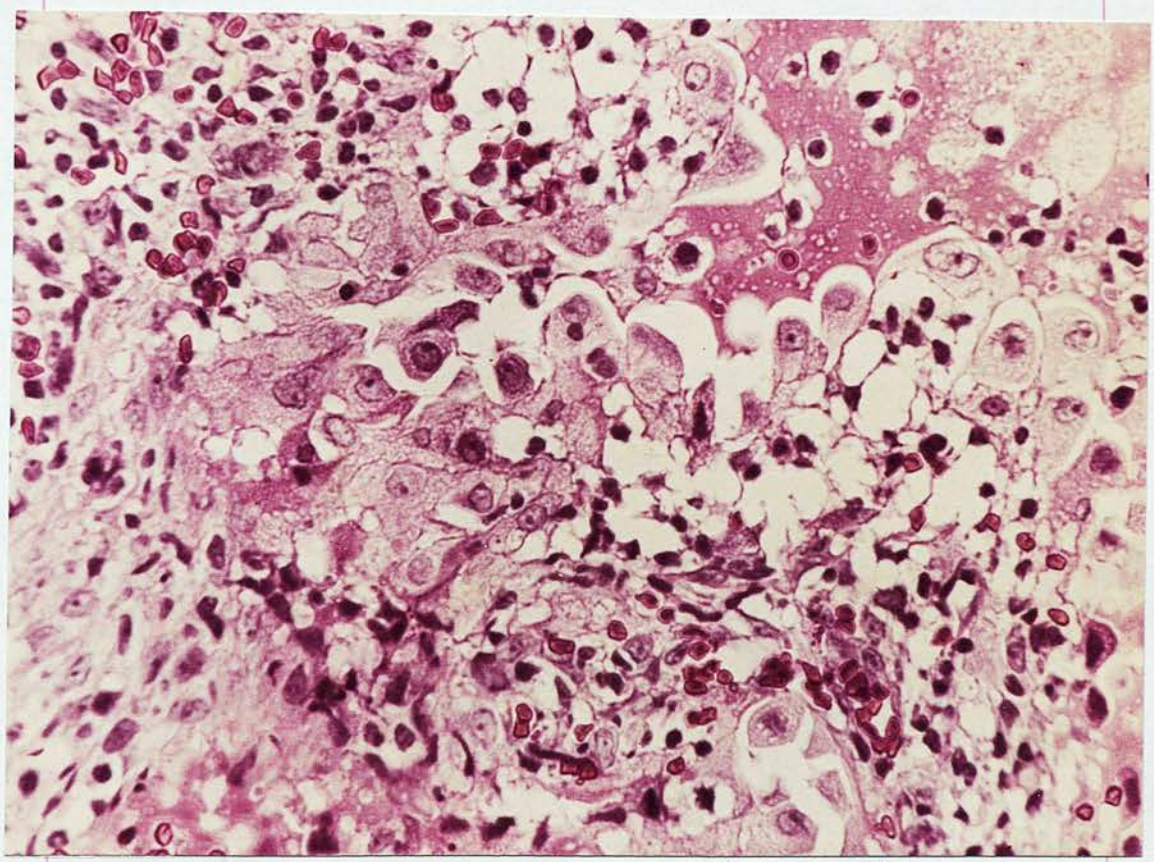


Fig. 69. (H.E. x 500). (Case IV)

Acute epithelial injury - desquamation and granularity of cytoplasm with irregular contours of cell outline.

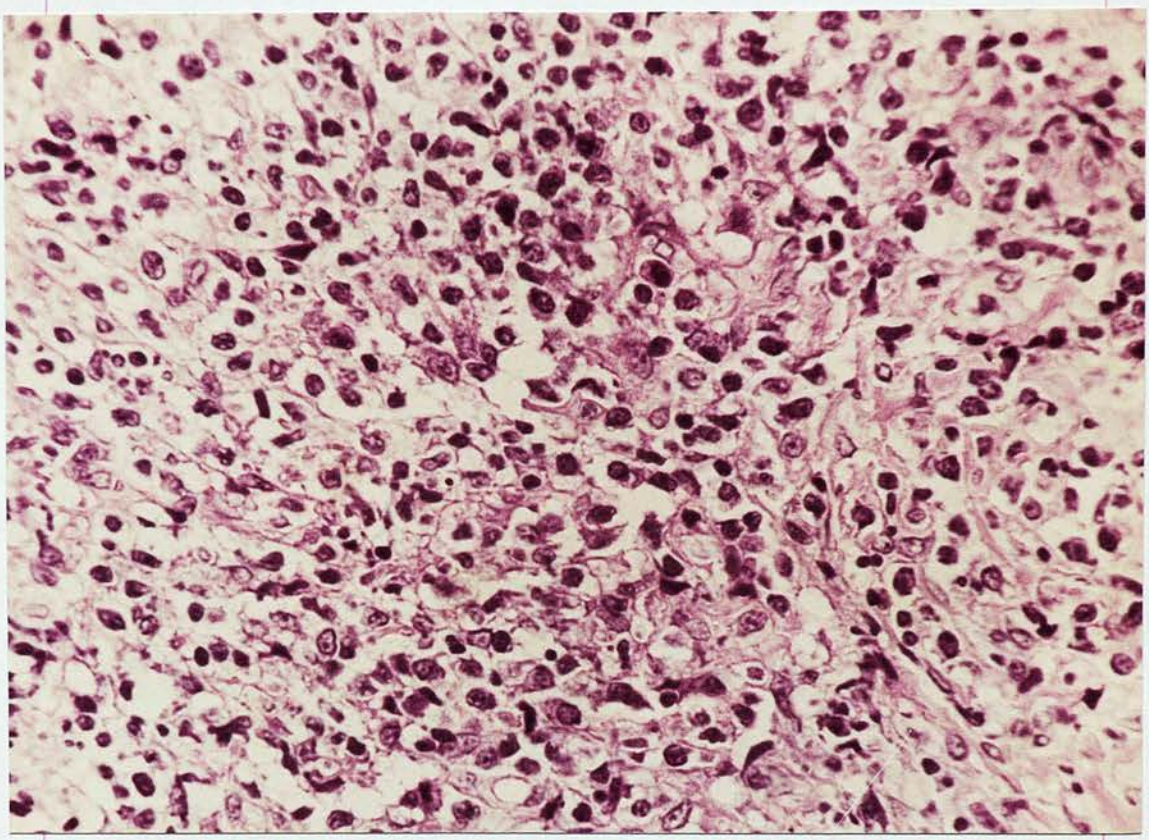


Fig. 70 . (H.E. x 500). (Case IV)

The "interstitial" exudate: Plasma cells, lymphocytes, macrophages and isolated epithelial cells. This is very similar if not identical with that seen in classical subacute thyroiditis.

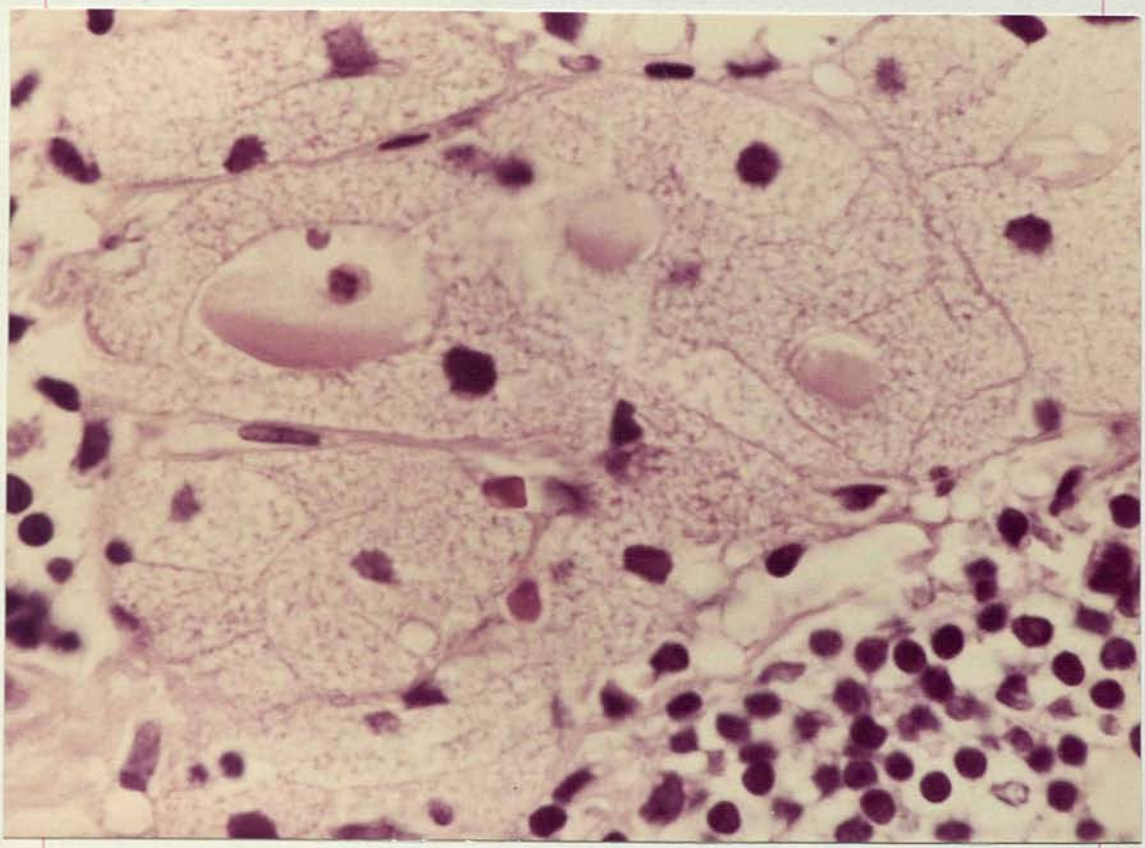


Fig. 71 . (H.E. x 1000). (Case IV)

Several small acini filled with colloid. The epithelium is very granular and closely resembles that seen in degenerate thyroid cells in tissue culture.

Case V.

Female aged 38. This patient complained of pain in the neck very soon after an attack of influenza. Biopsy was done several weeks after the onset of illness at a time when the patient seemed to be improving. No antibodies were found and the gland at operation looked normal.

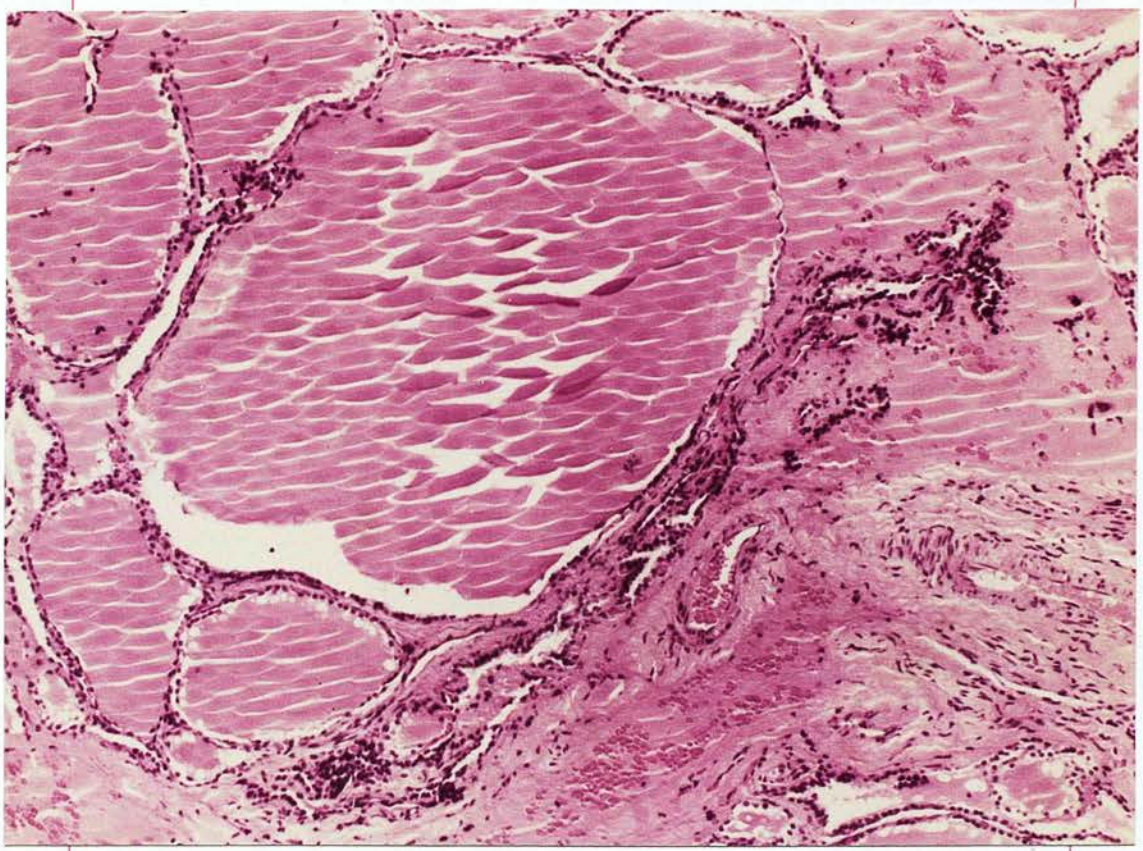


Fig. 72. (H.E. x 120). (Case V).

Small area of fibrosis with irregular cleft like spaces filled with pale colloid.

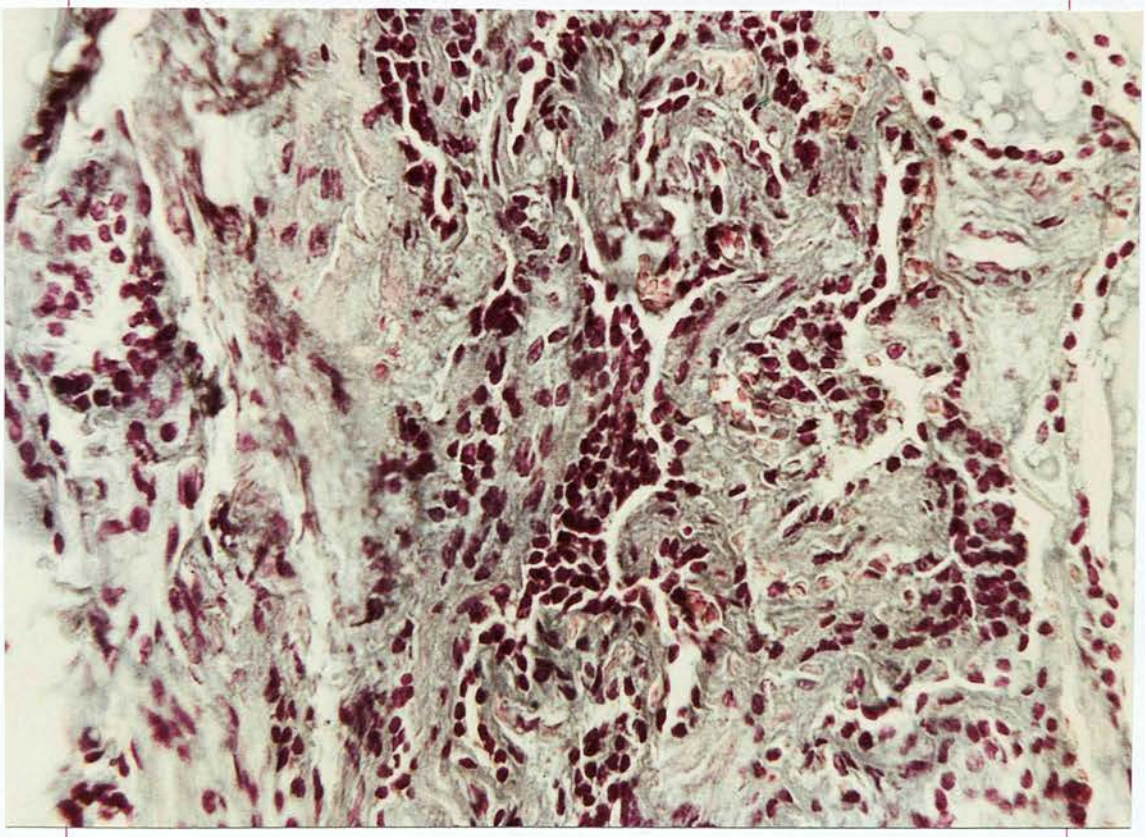


Fig. 73. (Masson trichrome x 500). (Case V).

Shows fibrosis and regenerating follicles lined by a single row of flat epithelial cells containing prominent nuclei.

Case VI.

Young man. No case history. Biopsy taken in the Middle East in 1928.

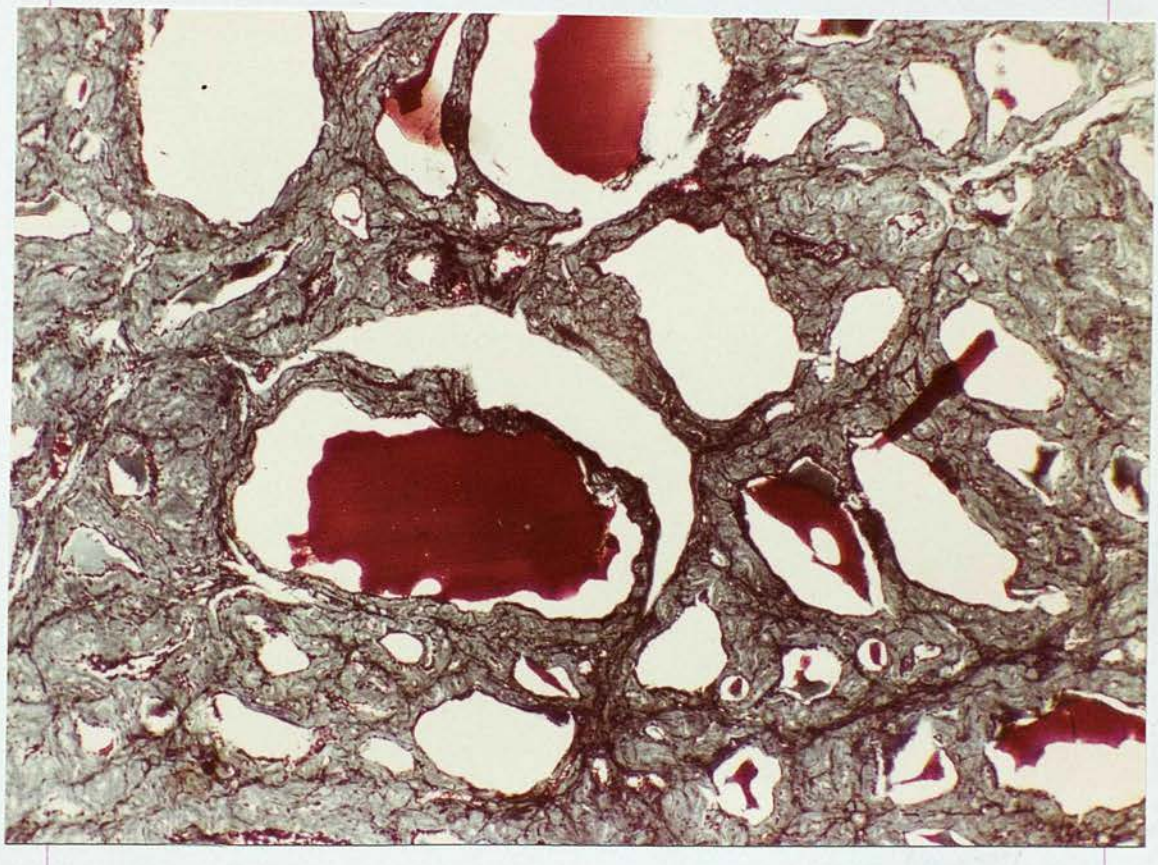


Fig. 74 . (Masson trichrome x 70). (Case VI)

Note the very curious interstitial fibrosis and flat insignificant epithelial lining of the follicles. The antecedent pathology can only be guessed at and the possibility of a healed subacute thyroiditis is not improbable.

Case VII.

No histology: Female aged 33 years complained of a tender, diffuse enlargement of thyroid. She responded to treatment with cortisone but relapsed several months later when the condition recurred. It subsequently improved and when last seen three months later she was in normal health.

She had a high antibody titre during the phase of clinical illness and this subsequently returned to zero.

Discussion of Pathology:

The microscopic appearances in cases I and II are acceptable to most pathologists as examples of the de Quervain type of subacute thyroiditis and cases V and VI are in keeping with the healed phases. Cases III and IV however, offer considerable difficulty and it may be that this group of 6 cases are not in fact homogeneous. In neither of these cases were the typical giant cells found but on the other hand the interstitial inflammatory tissue showed a similar if not identical cytology as that seen in more classical instances of the disease. The presence of a rather mucoid looking tissue,which contains isolated epithelial cells and macrophages rich in P.A.S. staining granules,is characteristic of subacute thyroiditis. The basophilic mucoid character of the interstitial tissue and the P.A.S. positive granules have been commented on but once in the literature (Lindsay and Dailey, 1954) and the present findings fully support the observations of these two authors. Although this series of cases is too limited to permit any dogmatic conclusions it is perhaps permissible to say that the findings of these changes should suggest the diagnosis of subacute thyroiditis

even in the absence of giant cells.

The prognosis in subacute thyroiditis appears to be good. The natural course of the disease is towards spontaneous recovery. In the great majority of reported cases the patient has been left with normal thyroid function. This finding is in marked contrast to Struma lymphomatosa where hypothyroidism commonly occurs. Hypothyroidism has, however, been reported as a sequel to subacute thyroiditis on more than one occasion. Lindsay and Dailey (1954) noted this complication in three of twelve cases in their non operative group.

Fibrosis was marked in several cases in the present series and if these changes are representative it seems most unlikely that the gland can ever return to normal. The fibrosis in subacute thyroiditis resembles that of scar tissue and is quite unlike the process of fibrous replacement of lymphoid tissue seen in Struma lymphomatosa. Furthermore the present study reveals an absence of "fuchsinophilic collagen" in subacute thyroiditis whereas this atypical material is commonly found in Struma lymphomatosa. It is of historical interest to note that

Professor Wegelin described the microscopic findings of De Quervain and Giordanengo's fourth case as those of Riedel's fibrosis! Unfortunately the classic descriptions of Riedel have delayed recognition of the fact that fibrosis of the thyroid may be reached by several quite different thyroid diseases.

Chapter IV. (Section II).

Immunology of Subacute Thyroiditis.

Material and Methods.

Six of the seven cases were tested for thyroid antibodies using the tanned cell haemagglutination and precipitin methods. All were tested during the time of clinical illness and were subsequently retested several weeks later when the patients were well again.

Results.

The results of the first tests are shown in Table VI, and of the second test, several weeks later, in Table VII.
(T.C.H. = tanned cell haemagglutination test).

Table VI

Case	T.C.H. titre	Precipitin test
1	80	-
2	2560	-
3	zero	-
4	160,000	-
5	zero	-
6	not done	not done
7	over 1024	+

Table VII.

Case	T.C.H. titre	Precipitin test
1	not done	not done
2	-	-
3	-	-
4	-	-
5	-	-
6	not done	not done
7	-	-

Discussion.

The relative values of the tanned cell and precipitin tests: Table I shows that four out of six cases gave positive results for thyroid antibodies with the tanned cell test whereas only one case had a positive precipitin test. This difference is adequately explained by the extreme sensitivity of the former method. It is doubtful, however, if the finding of thyroid antibodies in this condition is of any diagnostic value since the clinical and histological aspects of the disease are so distinctive.

Transience of auto-immunisation: A striking feature is the disappearance of antibodies shown

(Table II) by retesting several weeks later. The Lancet (1957) suggested that once the antigen-antibody reaction was initiated a self perpetuating mechanism might be set up whereby the gland was progressively destroyed. Three of the cases described here show that this is not the case and this finding is in agreement with the only report in the literature (Felix-Davies, 1958), where a progressive fall in the patient's antibody titre was noted.

Two of the patients gave negative results and the explanation is that either the initial injury to the gland was slight,or more likely the sera tested were taken at a late stage in the illness by which time all antibodies had disappeared.

Chapter IV. (Section III).

RIEDEL'S STRUMA.

In no other aspect of pathology have so many ignored an author's original description and used his name to illustrate entirely different disorders. Riedel (1896, 1897, 1910) described three cases of iron hard goitre occurring in a female and two males. All patients suffered from severe pressure symptoms and all showed dense leathery adhesions around the thyroid which made extirpation impossible. The histological findings in the first two patients are described in his own words. "If you look at the microscopic preparations you will find collections of round cells interspersed among normal thyroid tissue whereby the latter has been more or less destroyed. By just looking at the preparations one has no idea of how hard the tumour is; one expects solid fibrous tissue to constitute the tumour but as has already been said one finds only embedded round cells." Curiously enough Joll (1939) states that Riedel's disease is characterised by dense adult connective tissue and an absence of lymphocytic infiltration. The third case on microscopic examination showed both spindle and round cells. In his third publication (1910) he sums up the situation as follows.

"Typical case, young person, subacute swelling and hardening of the thyroid gland, directly severe dyspnoea: no fever so that infection is out of the question. The microscopic picture bears this out: there is an accumulation of spindle and round cells, among the normal elements of a goitre displacing the latter more and more; slight endarteritis as in many chronic inflammatory processes." He suggests the rarity of the disease is explained by spontaneous healing, compares the rapid development of young connective tissue to scar formation in a wound and concludes that the whole disease is puzzling.

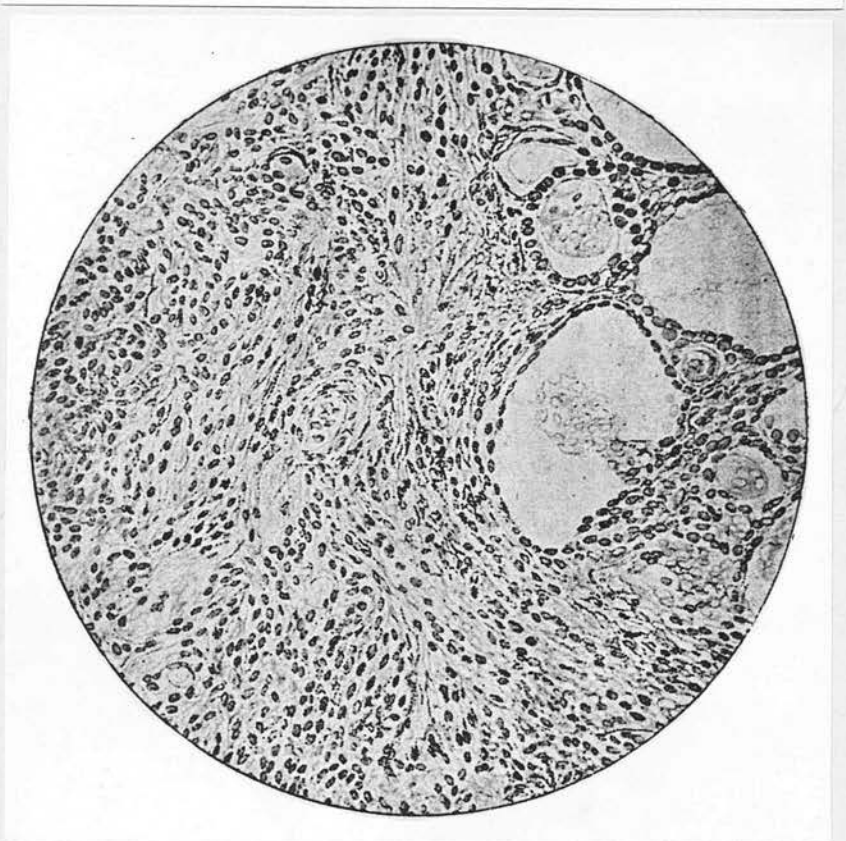
It is clear that the diagnosis of Riedel's struma demands the presence of dyspnoea, dense adhesions at operation and a rather variable histological picture showing normal acini, dense round cell infiltration and fibroblastic connective tissue. Several cases were published before 1920 which were in agreement with these findings. For example Karl Spannaeus (1910) reported a typical case in a man of 32 and gave a useful survey of the Continental literature. His coloured drawing of the biopsy shows young granulation tissue composed of fibroblasts and numerous round cells. In 1912 G.R. Murray and F.A. Southam gave

a clear description of "ligneous thyroiditis" in a male aged 23 years. Their patient had a hard goitre and "respiration was attended with great difficulty so that at night he was unable to lie down." At operation it was noted that the glandular tissue, capsule and adjacent structures were converted into dense fibrous tissue so hard that it was difficult to cut with a scalpel. Following operation their patient rapidly developed myxoedema but remained well on thyroid medication. In 1922 Ewing described 4 cases of goitre which showed a spectrum of changes ranging from lymphoid proliferation to fibrous overgrowth. He stated, accordingly, that Riedel's struma was the late stage of Hashimoto's disease and thus arose the "unitarian concept". Henceforth, so much licence was taken with the first description by Riedel that the original concept entirely lost its significance. The terms Riedel's struma and Hashimoto's disease were used interchangeably and failure to recognise the fibro-lymphoid phase of Hashimoto's disease led to its erroneous description as Riedel's fibrosis. Riedel described dense fibrosis outside the gland and not inside it. Repeatedly one finds in the literature so called cases of Riedel's struma

where the gland was fibrous, the patient had no pressure symptoms and the surgeon did not complain of dense adhesions, and yet the eponymn was applied despite the fact that Riedel did not describe dense intra thyroid fibrosis. The extent of the controversy on the identity or otherwise of Riedel's and Hashimoto's struma is illustrated in the appendix, which lists authors who believed the two diseases were related and those who did not.

The disease is rare and J.A.L. Clark points out that in "1008 consecutive thyroidectomies carried out in Edinburgh under the charge of Mr. K. Paterson Brown between 1943 and 1957 no case satisfied the clinical or pathological requirements necessary for classification under this title, although on a number of occasions such a diagnosis was erroneously made by the pathologist."

No acceptable cases of Riedel's struma could be found for inclusion in this study, and accordingly, no information can be given on the role of thyroid antibodies in this disease. A special plea has been made for the recognition of a fibroid phase in lymphadenoid goitre which should be clearly distinguished from Riedel's struma.



Reproductions of Riedel's original illustrations.

Chapter V.

The Significance of Basement Membrane Changes in Thyroid Disease.

Introduction:

In 1957 Roitt and Doniach demonstrated the presence of thyroid antibodies in Hashimoto's disease and suggested the stimulus for their production was the slow liberation of colloid from the thyroid follicles. More recently similar antibodies have been described in thyrotoxicosis (Goudie et al, 1957) and subacute thyroiditis (Felix-Davies, 1958). It appears that thyroglobulin is immunologically inert when contained within the follicle but is treated as a foreign protein by the host when extravasated into the interstitial tissue. The reason for leakage of colloid from the follicle is not understood but may be analagous to the escape of protein through the damaged glomerular basement membranes of nephritis. Accordingly, the purpose of this chapter is to describe the basement membrane changes in excised glands from patients with high thyroid antibody titres and determine what relationship, if any, exists between these two events.

Material and Methods:

Type of material:

1. Thyrotoxicosis: 8 glands from patients

with high titres were studied histologically and likewise 10 glands from patients with zero titres served as controls. At least 4 blocks were taken from each gland and the sections were graded on the degree of basement membrane injury without knowledge of the antibody titres.

2. Hashimoto's disease: 10 glands all showing diffuse Askanazy cell change, lymphoid follicles with interstitial round cell infiltration and varying degrees of fibrosis were studied.

3. Subacute or de Quervain's thyroiditis: Three typical cases were available for investigation.

Histological methods: Routine paraffin sections of tissues fixed in formol corrosive were used. The sections were stained with a modified silver stain (Slidders and Lendrum, 1958). This method was selected for use after trial of many other basement membrane stains since it gave clear and constant results.

Serological methods: Antibodies were detected by the tanned cell haemagglutination technique using human Gpo cells and purified thyroglobulin. The end point was taken as the last tube to show a broad even carpet of agglutinated cells. The term

"high titre" in this chapter means a serum titre of over 1 in 5000.

Results:

The normal appearance of basement membranes is seen in glands with zero antibody titres (fig.75). The membrane stains intensely and evenly; it measures approximately 0.1 to 0.2 μ in width and is closely applied to the base of the epithelial cells. It forms a continuous unbroken lining and completely seals off the follicle from capillary vessels. The latter are ovoid or polygonal spaces (fig. 76), bounded by the basement membrane whose inner surface contains flattened endothelial cells. Even in hyperplastic papillary processes the basement membrane zealously follows the proliferative epithelium.

Thyrotoxicosis: In this series abnormal membranes are seen mainly in glands with a high antibody titre. Table VIII shows the result of grading the 18 thyrotoxic glands on the degree of basement membrane injury. The injury is assessed as minor or absent (-), moderate (+) and severe (++).

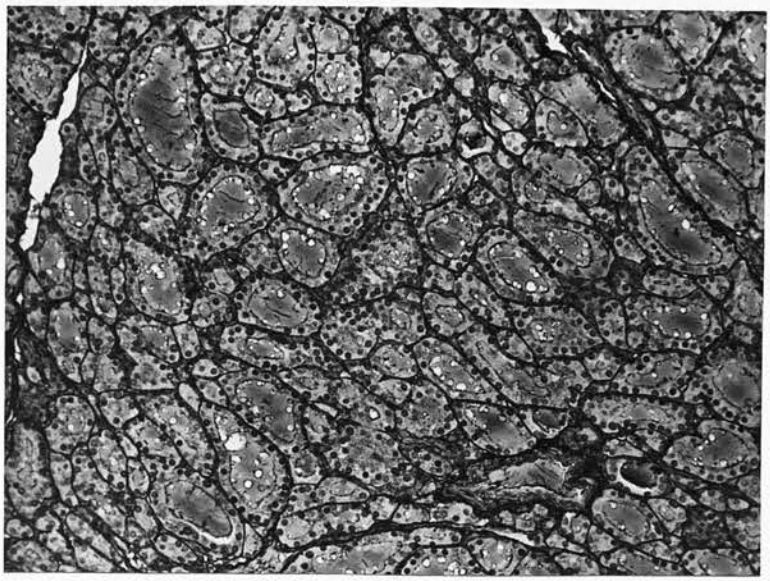


Fig. 75. (Silver x 135).
Normal basement membrane.

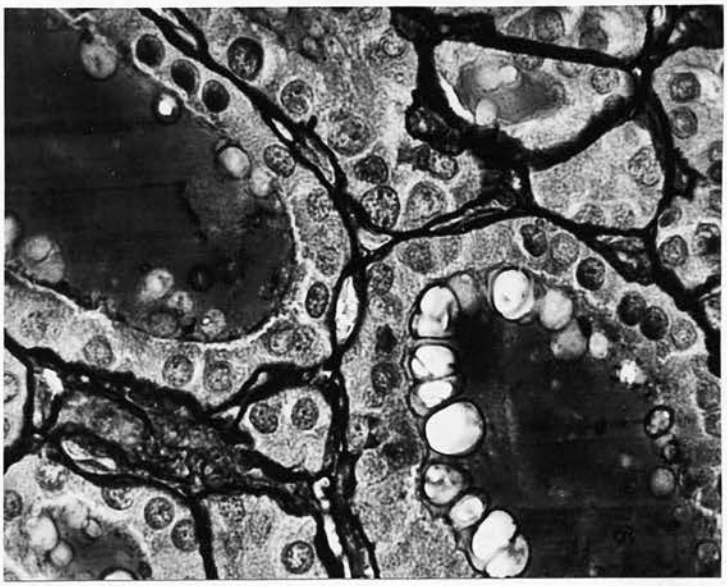


Fig. 76. (Silver x 650)
Normal basement membrane.

Table VIII.

Thyrotoxicosis. Zero antibody titre.	Thyrotoxicosis. High antibody titre.
-	++
-	++
-	++
-	+
-	+
-	+
-	-
-	
+	
++	

This table shows that eight of ten cases with zero titres showed no significant degree of membrane damage whereas six of seven patients with high titres showed extensive areas of injury.

The damage is entirely focal and consists of fragmentation, beading and duplication of the basement membrane. Fragmentation is seen as sharp breaks in continuity and is frequently associated with a wavy,irregular appearance (fig. 77) quite unlike the straight, smooth line of a normal membrane. Fragmentation must be distinguished from artefact caused by a faulty block or knife.

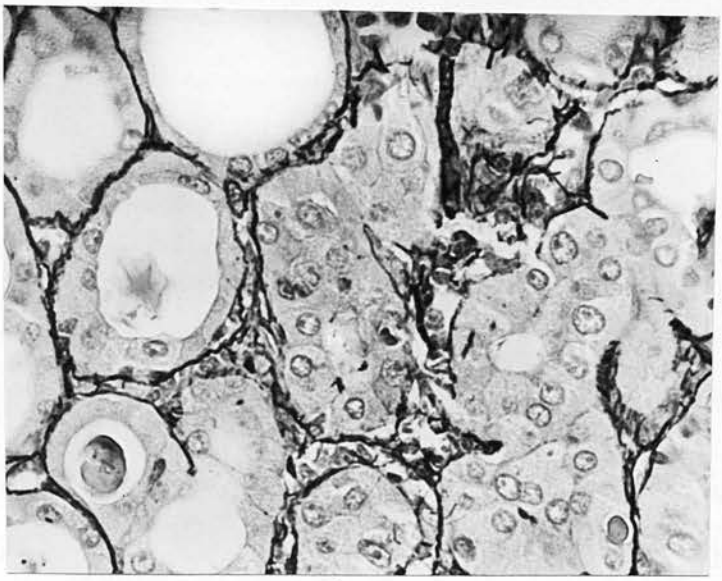


Fig. 77. (Silver x 500).

Fragmentation of basement membrane in thyroid gland.

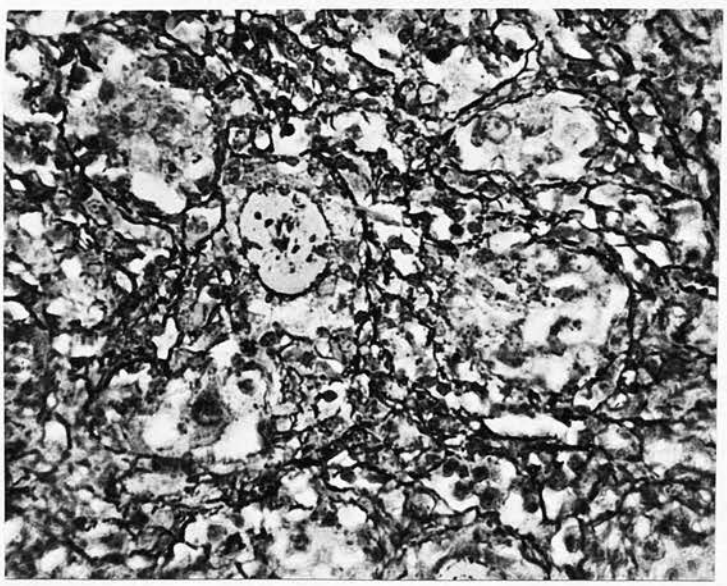


Fig. 78. (Silver x 400).

Lymphadenoid goitre: Typical beading and fragmentation.

Artefact appears as parallel rows of argentophil fibres attached at right angles either to a basement membrane or the interlobular fibrous tissue, resembling the teeth of a garden rake.

Beading is common. The term is borrowed from Sommers and Meissner (1954); it appears as minute argentophil dots or globules and is probably caused by failure of adjacent parts to stain with silver.

In duplication the basement membrane is split into two or more layers and this is seen more often in subacute thyroiditis than either Hashimoto's disease or thyrotoxicosis. Combinations of all three types of damage are usually present together.

Hashimoto's disease: The changes are qualitatively similar to those in thyrotoxicosis, but are diffuse and more severe. Fig. 78 shows typical widespread changes which include severe fragmentation. The basement membrane damage is not always as marked as this and Fig. 79 shows an apparently normal pattern,but the typical fragmentation and irregularity can be seen under a higher magnification in Fig. 80 .

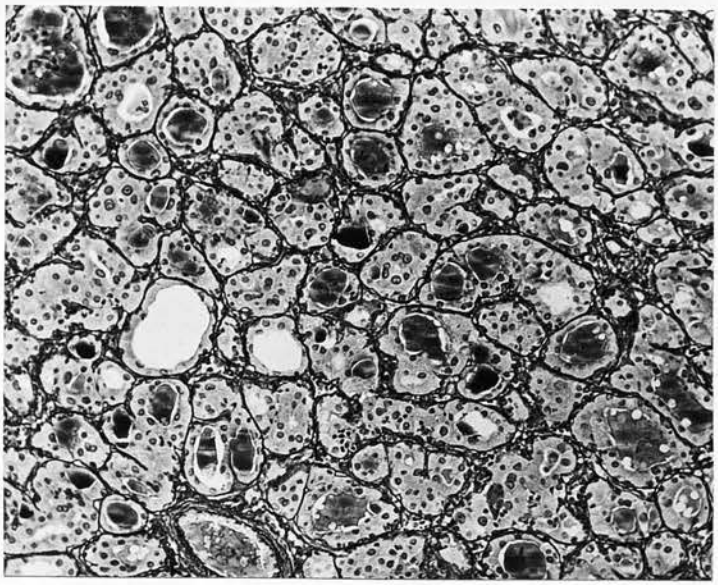


Fig. 79.

Lymphadenoid goitre: Apparently normal basement membrane but see higher magnification in fig. 80.

Fig. 80. (Silver x 625).

Fragmentation of basement membrane. See fig. 79.

All cases of Hashimoto's disease in this series had very high antibody titres and all showed severe diffuse injury to the basement membrane.

Subacute thyroiditis: The appearances are rather different here where there occurs an increase of reticulin-like fibres between follicles, and the fragmentation seems much coarser (Fig. 81) than that previously described. Follicles showing giant cells or swollen epithelium usually display marked duplication of the membrane whereas intact and normal looking follicles show no change.

Only one of the three cases showed an appreciable titre of antibody although all showed quite severe basement membrane changes.

Discussion:

In most instances damage to basement membranes was closely associated with a round cell infiltration composed of lymphocytes and plasma cells and Fig. 80 shows plasma cells intimately associated with a breach in the follicle. In a few glands extensive membrane damage was seen in areas which did not contain chronic inflammatory cells and this finding agrees with that of Sommers and Meissner (1954) who pointed out that "basement

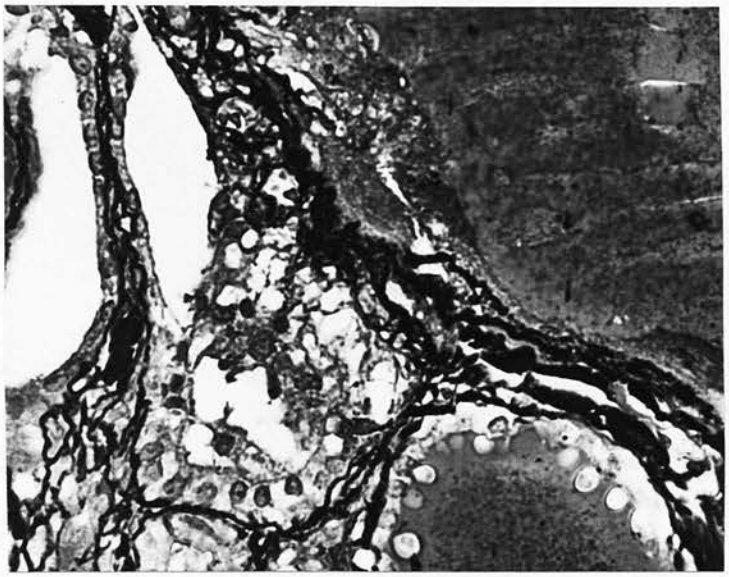


Fig. 81. (Silver x 500).

Subacute thyroiditis: Duplication and coarse fragmentation.

membrane changes need not be associated with lymphocytic nodules."

The present findings establish a clear relationship between basement membrane damage, as shown by silver impregnation methods, and the presence of antibodies in significant titre. Admittedly, most silver staining methods are notoriously fickle and the problem of artefact is serious. Nevertheless in our experience this new modified silver method is remarkably free from the usual defects.

The possibility that the fragmentation may be an artefact due to infolding of the thyroid follicle in a particular plane of section was excluded by the critical examination of thyroid tissue in serial section.

The cases of thyrotoxicosis were chosen for their very marked difference in antibody titre. In one group the titres were high and in the other no antibodies were present. This method was the only way in which the relationship between antibodies and histological changes could be established since the intermediate group of glands gave great difficulty in classification.

Accordingly, these findings appear to

establish a relationship between irregularity and lack of continuity of the follicular basement membrane and the escape of colloid into the interstitial tissue with consequent inflammatory cell reaction. Thus there would seem to be a case for regarding basement membrane damage as the anatomical basis of thyroid antibody formation.

The state of the basement membrane in epithelium showing Askanazy cell change is of special interest since this type of epithelium is characteristic of Hashimoto's disease. Askanazy cell change in the present study was nearly always associated with lymphocytes and plasma cells but in two instances there was no significant degree of round cell infiltration present and in both of these cases normal basement membranes were seen. This finding suggests that the nutritional change leading to the Askanazy type epithelium, does not necessarily result in impairment of the basement membrane.

The factors responsible for basement membrane damage remain obscure although in some instances e.g. a virus induced thyroiditis, it is probably secondary to severe epithelial damage. If the basement membrane deficiency in other forms of

thyroiditis is secondary to epithelial injury it would then seem reasonable to ascribe it to the action of thyroid antibodies which are assumed (Editorial B.M.J., 1958) to be responsible for the parenchymatous damage in Hashimoto's disease. However it is difficult to believe that these circulating antibodies possess any significant cytotoxic effect because they are present in high titre in some cases of thyroid hyperfunction. In such instances one would expect their cytotoxic effects to result in suppression of function. Accordingly it seems probable that the antibodies are related with the phagocytosis of extravasated colloid and that the primary defect in Hashimoto's disease is a failure to maintain the integrity of the basement membrane as Sommers and Meissner (1954) suggested.

In Hashimoto's disease and thyrotoxicosis there is a reasonably good correlation between basement membrane pathology and the presence of antibodies although admittedly exceptions were found. The relationship is not so definite in subacute thyroiditis and perhaps a larger series of cases is required to elucidate this point.

Summary and Conclusions.

1. Basement membrane changes are widespread and severe in Hashimoto's disease and subacute thyroiditis. Similar changes are seen to a lesser degree in thyrotoxicosis

2. Basement membrane damage is usually associated with lymphocytic and plasma cell infiltration but may be seen independently, possibly because it occurred at an earlier stage of the disease.

3. In this series of cases there is a direct relationship between basement membrane irregularities and high antibody titres.

4. If the function of the basement membrane is to maintain the immunological integrity of the follicle by preventing access of colloid to the interstitial tissue, solution of the continuity of the membrane as observed in this series provides an anatomical basis for thyroid antibody formation.

Final Abstract and Conclusions.

Serum from 142 patients suffering from diverse thyroid diseases has been examined for thyroglobulin antibodies. Another 148 patients without clinical evidence of thyroid disease and 50 healthy blood donors served as controls. The sera were examined by the highly sensitive tanned cell haemagglutination technique and the relatively crude precipitin method.

Antibodies were found in 20/22 cases of lymphadenoid goitre, 16/19 cases of myxoedema, 4/9 cases of thyroid carcinoma, 34/74 cases of thyrotoxicosis, 4/34 cases of simple goitre and 4/6 cases of subacute thyroiditis. The finding of antibodies in such diverse conditions means that antigenic colloid has been liberated from the thyroid follicles in sufficient quantity to provoke an antibody response. The antibodies are not necessarily cytotoxic and part of their function is to aid the phagocytosis of unwanted colloid. Therefore it seems likely that autoimmunisation is a biological response and is not characteristic of any particular disease.

The chief value of serological methods is their helpfulness in the clinical diagnosis of lymphadenoid goitre where the precipitin test is

of greater value than either flocculation tests or estimation of serum gamma globulin. Nevertheless positive precipitin tests may occasionally be found in thyroid carcinoma and undue reliance should not be placed, at the present time, on the precipitin test which should be assessed with both clinical and biochemical findings.

The histological findings in glands removed from patients with antibody titre are variable but on the whole lymphocytic and plasma cell infiltration is a prominent feature. Severe degenerative changes are found in the basement membrane of thyroid follicles in such instances and this abnormality precedes the inflammatory cell reaction.

The pathology of 100 cases of lymphadenoid has been reviewed and the author unreservedly supports the view that this is a progressive disease which may ultimately lead to severe fibrosis and myxoedema. Two new histological findings are described. Firstly, widespread basement membrane damage is a striking and constant feature and is an integral part of the pathological picture. Secondly, a curious fuchsinophilic material is frequently found in the lymphoid follicles of lymphadenoid goitre. The chemical nature of this material is quite unknown but it may possibly

represent gamma globulin.

Although lymphadenoid goitre is traditionally regarded as eminently benign, there have been reported in the literature several instances where malignancy is also present. This relationship between lymphadenoid goitre and thyroid carcinoma is illustrated by a further two cases and attention is drawn to three patients all of whom suffered from lymphadenoid goitre and all of whom succumbed to a malignant reticulosis. It is concluded that the association of lymphadenoid goitre and malignancy is not entirely fortuitous.

The criteria for the diagnosis of Riedel's struma are discussed and it is emphasised that the diagnosis should not be made on histo-pathological grounds alone. A special plea is made for the differentiation of Riedel's struma from the lymphofibrous phase of lymphadenoid goitre.

A short historical account is given of sub-acute thyroiditis and the clinical, histological and immunological features are discussed. A curious finding is the presence of fibrosis in biopsy specimens of thyroid removed at a time when the clinical features suggest that only acute inflammatory changes would be present. It is

concluded that serological methods play no part in the diagnosis of this disease.

APPENDICES (TECHNICAL METHODS)

APPENDIX I.

Fuchsinophilic Material in Germinal Centres.

Histological Methods:

Routine paraffin sections of tissue fixed in formol-corrosive or Zenker were used. Formol saline and Bouin's fixative give poor results.

The staining method found most satisfactory is as follows -

1. Bring sections to water and stain in Celestine Blue (.5% in iron alum) for 10 minutes.
2. Mayer's haemalum - 10 minutes.
3. Differentiate in acid alcohol and blue in dilute ammonia.
4. Wash well in water.
5. Stain in fresh solution of 1% acid fuchsin dissolved in distilled water.
6. Rinse and differentiate in 1% phospho-tungstic acid.
7. Dehydrate in alcohol and clear in xylol.
8. Differentiate further in aniline-xylol until fuchsin-material just begins to lose its bright red colour.
9. Counterstain in 0.5% auramine in xylol.
10. Rinse in xylol and mount in balsam.

APPENDIX II.

Riedel's struma and Hashimoto's disease are distinct and separate conditions.

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Riedel's struma and Hashimoto's disease are related conditions.

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APPENDIX III.

Tanned Cell Haemagglutination Method.

Refs: "The absorption of proteins on erythrocytes treated with tannic acid and subsequent haemagglutination by anti-protein sera."

(1) S.V. Boyden, 93, 1951, PI07. J. E.M.

(2) Stavitsky, A.B., J. Immunol. 72: 1954.

Principle:

Treatment of human GpO erythrocytes with suitable concentrations of tannic acid renders them capable of absorbing certain protein molecules from solution in saline. Red cells treated in this way and washed are agglutinated by the homologous anti-protein sera. Thus sera can be titrated for antibodies against antigens absorbed on the cells exposed to tannic acid; furthermore, small amounts of antigens can be detected through their power to inhibit haemagglutination of the treated cells.

Materials and Methods:

(1) 4% suspension of washed human GpO cells:

Human GpO blood preserved in A.CD is kept at 4° C. and may be used up to 3 weeks. For use an aliquot is taken, washed in saline 3 times and re-suspended to give a 4% solution in saline pH 7.2.

(2) Preparation of erythrocytes with tannic acid.

To a 4% suspension of washed cells add an

equal volume of tannic acid strength 1/20,000 diluted in saline pH 7.2. Stand at room temperature for 30 minutes, centrifuge GENTLY and wash three times in buffered saline, and resuspend to give a 2% suspension.

Absorption of Antigens:

An equal volume of suitably diluted antigen (usually 1/20 - 1/50)* is added to the 2% solution of red cells and the mixture allowed to stand at room temperature for 30 minutes. The sensitised cells are now washed three times with 1/200 normal rabbit serum.

* (If pure thyroglobulin is used then 1/1000).

Note: When using a fresh batch of antigen make a 1/25 dilution and at the end of 30 minutes incubation, test for agglutination as follows. Place 0.2 cc. of the mixture in a small test tube and centrifuge slowly for 3-4 minutes. Remove the tube and examine the sedimentation pattern. If no agglutination has occurred the red cells will form a button at the bottom of the tube. If a 'carpet' or 'ring' has formed then the antigens must be diluted further, e.g. 1/40 and the procedure repeated. The sensitised cells are now washed three times in 1/200 normal rabbit serum. Prior to use this rabbit serum has been inactivated

at 56°C. for 10 minutes and then absorbed with its own volume of washed (x3) packed human cells for 30 minutes at 37° C.

The cells are resuspended in 1/200 NRS in saline pH 7.2 to give a 1% suspension.

Exposure of cells to test serum:

1. Set out 2 rows of 11 tubes for each test serum; place 0.1 ml. serum diluted 1/10 in the first tube of each row and thence-forth run doubling dilutions to a titre of 1/10,000.

2. To the front row add 0.1 ml. of tanned antigen coated cells. To the back row add 0.1 ml. of tanned cells.

3. Saline Controls: In two tubes place in each 0.1 ml. tanned antigen coated cells and 0.1 ml. saline; in another two tubes place in each 0.1 ml. saline and 0.1 ml. of tanned cells.

4. With each batch of tests always put up a negative serum and a positive serum.

Shake all racks vigorously and leave at room temperature for 2-4 hours. Take final readings in the morning.

Important Practical Points:

Tannic acid:

A stock solution of 1 gm. in 200 cc. of

buffered saline pH 7.2 may be kept at 4°C for not more than 3 weeks.

Cells treated with tannic acid must be washed three times to remove excess acid which can act as amboceptor and causes haemolysis.

Red cells:

Cells stored in Alserver's solution at 4°C may be used for a period of 3 weeks. If during preliminary washing excessive haemolysis is observed the cells must not be used.

Normal rabbit serum:

This prevents lysis and aids in the resuspension of red cells after centrifugations.

The rabbit serum should first be absorbed with packed GpO cells (if GpO cells are used) before use.

The possibility exists that the tanned cells treated with antigens may not be completely saturated with the specific protein and consequently might absorb proteins of the rabbit serum in which they are washed; it is also possible that rabbit serum proteins might replace some of the antigen molecules on the cell surface. Components of rabbit serum absorbed in this way would be available at the surface of cells to react with antibodies against them in the event of these being present in the test

serum and thus a possible source of error would be introduced. As a precaution against such errors it might be advisable in some cases, to wash only in a dilution of normal serum from the same species as that which is being tested. However, N.R.S. in a dilution of 1/200 has not hitherto given trouble when the test sera are of human origin.

Human plasma or serum 1/200 can be used instead.

Absorption of Antigens:

Every care must be taken to manipulate the cells GENTLY after exposure to antigen. It is absolutely essential to wash the cells thoroughly after exposure to antigens. Free antigen will completely inhibit the haemagglutination reaction.

Sera:

Test sera must be stored at $4^{\circ}C$ and are inactivated at $56^{\circ}C$ for 10 minutes before use. Sera which are turbid may be infected. Infected sera must not be used as bacterial haemagglutinations will interfere with the reaction. Therefore examine suspect sera under phase contrast (high magnification) for organisms.

Antigens:

Make quite sure that the concentration of antigen used does not by itself cause haemagglutin-

ation.

Saline:

Phosphate buffer to pH 7.2.

Tubes:

3" x 3/8th".

APPENDIX IV.

Demonstrations of Thyroglobulin by a Modified Oudin Technique.

References: Oudin, J., L'Analyse immunochinique qualitative, methods par diffusion des antigens au sein de l'immunserum precipitant gelose. Ann. Inst. Pasteur. 75:30, 1948.

Oudin, C.L. and Fulthorpe, A.J.: Antigenic analysis by diffusion. J. Path. Bact.: 65:49, 1953.

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Materials:

Thyroid extract: Human thyroid gland obtained at thyroidectomy or from a fresh post-mortem is frozen out into 0.5-1 mm. slices with a razor blade put into 2-3 vols. of 0.9% NaCl at 2°C, left overnight and centrifuged using supernatent for the test. Dilutions of this antigen should be used for sera with low antibody content.

Clarification of Agar: 1 per cent w/v New Zealand agar is suspended in 0.9% NaCl and dissolved by heating in a boiling water bath. The solution is twice filtered rapidly through well rinsed glass wool in a Buchner Funnel to remove grossly insoluble particles. 1% sodium azide is added as a preservative.

To the glass wool filtered agar is added 1 or

2% w/v of a mixture of equal parts of powdered bentonite and 'Hyflo Super-Cel' (Johns-Manville, Co. Ltd.) and the whole shaken vigorously by hand to disperse the clarifying agents. The suspension is stored at 56°C for several days, the clarifying agents being resuspended daily by gentle inversion of the bottles. When the cloudy flocculum is completely carried down from the supernatent by the bentonite 'Super-Cel' mixture the clarified agar is carefully decanted. The clarified agar is filtered through a fluted Whatman No.5 paper in a heated funnel into a bottle standing in hot water. The first 25 ml. or so are returned to the funnel for re-filtering. It is stored in 30 ml. screw cap bottles. It is convenient to make 1 litre at a time.

Method:

Dissolve agar solution by heating the container in boiling water bath, then place in 42°C bath. Mix 0.2 ml. of serum with 0.2 ml. of agar solution in a 3 x $\frac{1}{2}$ tube at 42° and place the mixture at the bottom of a Widal tube with a long pasteur pipette at room temperature, taking care to avoid bubbles. When this layer is set, mix agar solution with an equal amount of normal saline at 42° - 45° and place 0.8 cm. column of this on the serum layer. When the second layer is set, add the antigen layer, consisting of equal amounts of agar

solution with thyroid extract.

The antigen and the antibody in the patient's serum diffuse slowly towards the neutral saline zone. A line of precipitation becomes visible when they meet in optimum concentrations. If the serum contains a large amount of antibody the precipitin becomes visible in the saline layer after 2-3 days and thickens as time goes on. When the antibody titre is low, the precipitate may take up to 14 days to become clearly visible, and is more diffuse.

As a quick screening test the precipitin test can be done in Durham tubes by layering the thyroid extract gently onto patient's serum. A thin white line appears after 10-20 minutes, when strong antibody is present. The Oudin technique has proved more sensitive.

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